



**PHD**

**Breeding System Evolution of Malagasy Plovers  
Natural Behaviours and Experiments**

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# **Breeding System Evolution of Malagasy Plovers**

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## **Natural Behaviours and Experiments**

**Jorge Enrique Parra Bastos**

A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department of Biology and Biochemistry

May 2014

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For my wife Marce and my daughter Emma with all my love.

## Summary

Breeding systems encompass a diverse range of mating and parental care strategies that develop in relation to reproductive success. However, which factors determine the reproductive strategy that will achieve success, in terms of Darwinian fitness, is still a controversial issue in theoretical and empirical behavioural ecology. I argue in this dissertation that animal reproductive strategies depend on variation in the social environment, which is influenced by the number of available mates in a population. The purpose of this thesis was therefore to investigate the role of mating opportunities, in breeding system evolution by testing current theoretical models. First, I studied different contributory factors that have been proposed to influence variation in mating opportunities, including, brood attendance (chapter two) courtship (chapter three) and pair bond stability (chapter three) in four closely-related plover species of genus *Charadrius*: Kentish plover, *C. alexandrinus*; Kittlitz's plover, *C. pecuarius*; Madagascar plover, *C. thoracicus*; and white-fronted plover, *C. marginatus*. Second, I investigated whether mating opportunities differed between these plover species that exhibit different mating and parental care patterns yet have similar life histories and ecology (chapter three). Third, I also studied the spatial movements of plovers when searching for a mate (chapter four). Consistent with theoretical models, mating opportunities were highly variable between species suggesting that mating opportunities are an important component of the social environment. Since mating opportunities are linked to operational sex ratios (OSR, the ratio of sexually active males to females), this suggests that OSR exhibits substantial variation among closely-related species that may influence the direction and intensity of courtship and mate searching behaviour (chapter three and four) and whether to care for or desert offspring (chapter two). These results from wild populations suggest that a demographic property of the populations, OSR, influences mating opportunities, and in turn, facilitate different intensities of sexual selection and parental care patterns.

# CHAPTER ONE

## **Introduction**

## **Thesis Outline**

Jorge E. Parra

### **Breeding systems**

Animal breeding systems involve all aspects related to mating and parental care patterns displayed by males and females of sexual organisms (Reynolds 1996). Mating patterns are described based on forms and duration of pair bond, number of mates, forms of courtship and mating resources defended or offered. Mating patterns also consider multiple mating by females, distinction between genetic and social partner and the potential of genetic benefits (Reynolds 1996). Thus, variation of sex roles on reproduction is diverse including monogamy (social and genetic) and polygamy mating patterns.

Parental care patterns are the result of parental investment which is the investment of the parent(s) in the offspring to increase their survival at the cost of the parent's ability to invest in additional offspring (Trivers 1972). Patterns of care have been described based on the form and duration of the parental investment provided by the male and/or the female to the offspring. For example, animals exhibit different parental care patterns including female-only care in the majority of arthropods, reptiles and mammals; male-only care in some species fish and anurans; and biparental care which prevails in birds (Balshine 2012, Trumbo 2012). Most of these parental care patterns match in some degree with mating patterns (Reynolds et al. 2002). For instance, about 90% of birds exhibit social monogamy and biparental care whereas about 90% of mammals exhibit polygyny and female-only care (Reynolds et al. 2002). Multiple life history traits also influence breeding systems. For example, if egg and juvenile mortality is high in the absence of care, selection would favour parental care (Klug et al. 2012). This has been observed in the offspring of different species of invertebrates that need parental care to survive (Clutton-Brock 1991).

Reproductive patterns, then, exhibit large variation at different taxonomic levels, and even at population level, in nature (Reynolds et al. 2002, Balshine 2012, Trumbo 2012, Vincze et al. 2013). The diversity of animal breeding systems has been of particular interest to behavioural ecologists because it is still controversial how natural and sexual selection has shaped different animal social behaviours and life-history traits related to reproduction.

### **Sexual selection and parental investment**

Sexual selection theory predicts that sex roles in reproduction are influenced by differences in parental investment, which in turn, explain the strength of sexual selection on the sexes (Darwin 1859, Trivers 1972, Andersson 1994, Davies et al. 2012, Alcock 2013). The differential initial investment in gametes size between the sexes, called anisogamy, has been suggested to influence the evolution of conventional sex roles (Trivers 1972, Clutton-Brock 1991). This implies that males should be more focused on mating because they invest less in generating high quantities of energy-cheap sperm; whereas females are predisposed to be the caring sex because they invest more in fewer, but energy-rich, eggs. As a consequence, if females provide most of the parental investment, they will be limited in the mating pool; and then, the strength of sexual selection should act on males, in the form of male-male competition and female choice for mates (Andersson 1994).

However, current work has pointed out that past investment (anisogamy) may not explain decisions about caring for or deserting the offspring (Kokko & Jennions 2008). That is, females would not be predisposed to post-zygotic care because of the high initial investment on eggs (concord fallacy; Dawkins & Carlisle 1976). In fact, producing energy-rich eggs by females is costly in terms of body condition, thus, females should not be selected to invest more in post-zygotic care than males (Kokko & Jennions 2008). In addition, sex roles are not fixed, and they are influenced by spatial and temporal variation of mate availability (Forsgren et al. 2004), as indicated by the operational sex ratio (OSR), the ratio of sexually active males to fertilizable females (Emlen & Oring 1977).

### **Recent theories of breeding systems evolution**

Current theoretical models suggest three key modifications to understand conventional sex roles in reproduction without assuming a trade-off between mating and parental effort, and sexual differences in mating and parental care investment in advance (Queller 1997; Kokko & Jennions 2008). First, sperm competition is predicted to play an important role in the investment of parental care (Queller 1997). In species with extrapair paternity and internal fertilization, mothers sire all her offspring in a breeding event whereas potential fathers no known the number of young sired in a breeding event. Consequently relatedness to the offspring is higher



## INTRODUCTION

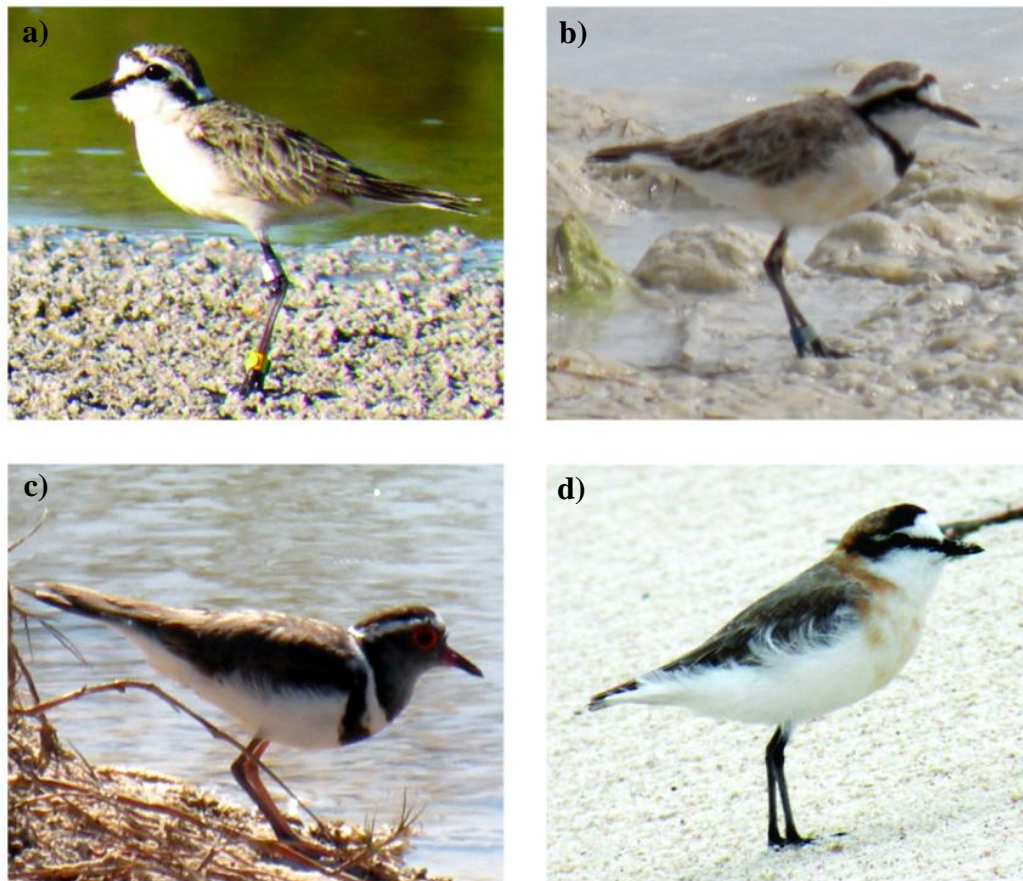
for females than males, which in turn, influence in least paternal care. In birds, for example, male food provisioning to the offspring decreases in species that exhibit a high frequency of extrapair paternity (Møller 2000).

Second, sexual selection on males decreases paternal care. Bias in the OSR can create strong sexual selection on the common sex, usually the male. Nonrandom variance in mating, then, increases the average expected mating success in some males over average males, such as in polygamous species. This means that mated males should not care for the offspring because they have high reproductive success at a male-biased OSR so that they are better off by competing for additional females. In contrast, for an average male is irrelevant care for young or not, because these males may not reproduce (Queller 1997; Jennions & Kokko 2010).

Finally, recent theoretical models propose that a key aspect of sex role evolution is adult sex ratio (ASR; Donald 2007); the ratio of adult males to females in a population. According to Fisher (1930), males and females should be produced at an equal number and any biases in the sex ratio will create a counteracting force to produce an unbiased sex ratio. That is, any biases in the sex ratio will create a shortage of one sex. This sex will increase its relative reproductive value in terms of number of mates because of the excess of the other sex. Then, selection will favour the parents that produce the rare sex (the counteracting force) in order to reduce the bias. However, ASR exhibits high variation among natural populations due to biased primary sex ratios at the time of conception, secondary sex ratios at birth, sex-biased dispersal and immigration, and sex-differential mortalities of adults, the tertiary sex ratio (Wolff et al. 2002; Clutton-Brock et al. 2002; Liker & Székely 2005); thus relaxing the assumption that ASR is even (Donald 2007). This is because if differential adult mortality generates biased ASR, frequency dependent sex allocation is not expected to produce even population sex ratio (Fisher 1930).

Furthermore, ASR and OSR are often assumed to be identical, although this assumption is not justified in many biological systems (Székely et al. 2014). For instance, in many species of mammals the OSR is strongly male-biased whereas the ASR is female-biased because male-male competition induces higher mortality and emigration in males, skewing the adult sex ratio toward females (Wolff et al. 2002; Clutton-Brock et al. 2002). In addition, whereas ASR is largely influenced by

demographic processes (e.g. mortalities, maturation), OSR is primarily the outcome of behavioural decisions whether an animal is (or isn't) available for mating (Székely et al. 2014). Recent studies also suggest that biased ASRs predict sex roles, mating systems and pair-bonds in birds (Liker et al 2013, Liker et al. 2014). Thus, when ASR shifts toward males, they should compete more intensely for females and provide more care for the young; and when ASR shifts toward females, they should provide more care for the young (McNamara et al. 2000; Kokko & Jennions 2008; Jennions & Kokko 2010). Therefore, ASR, as a demographic property, is an important component of sex roles in reproduction.



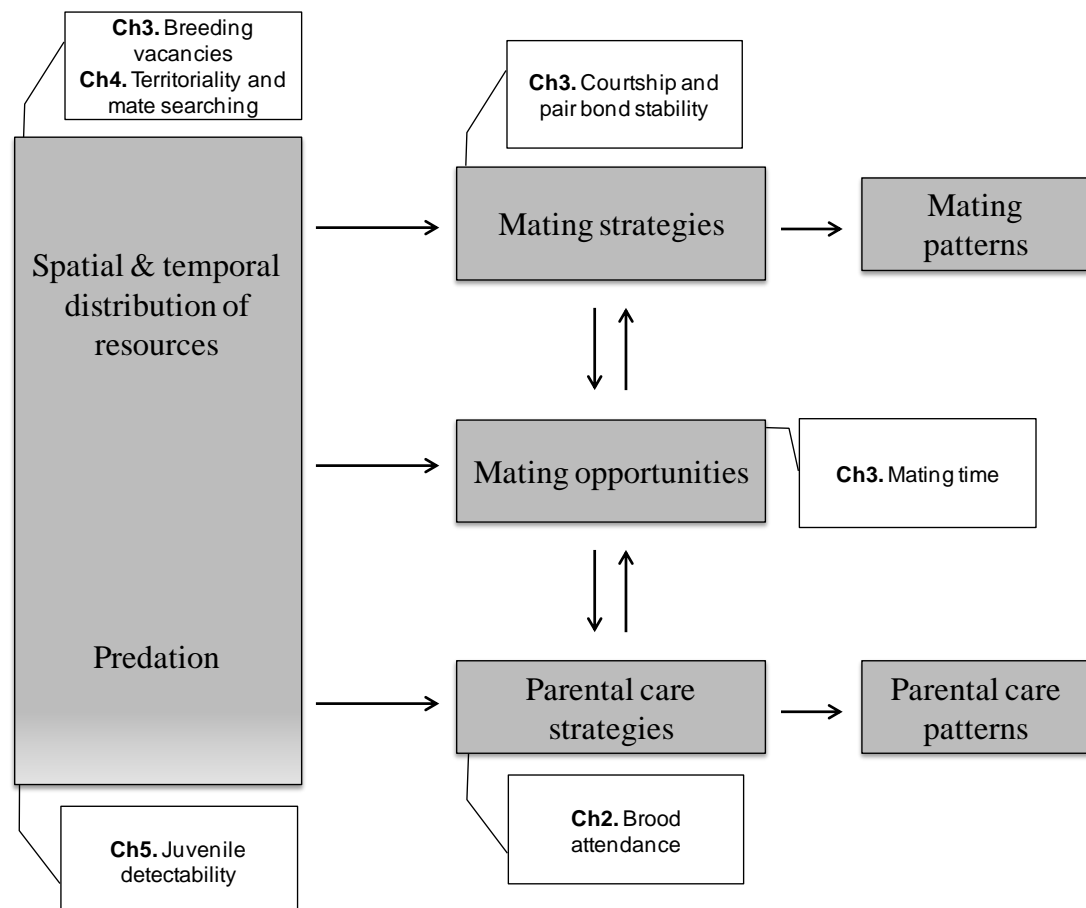
**Figure 1.** Four sympatric breeding species of Madagascar: (a) Kittlitz's plover, *Charadrius pecuarius*; (b) Madagascar plover, *Charadrius thoracicus*; (c) Three-banded plover, *Charadrius tricollaris*; (d) White-fronted plover, *Charadrius marginatus* (credit: Jorge E Parra).

**Malagasy plovers: model species to study breeding systems**

Shorebirds (sandpipers, gulls, plovers and allies) are a diverse group with almost 385 species distributed globally (Gill & Donsker 2014). They have a diversity of life histories and adaptations to live in wetland, coastal and inland habitats. Shorebirds have a remarkable diversity of breeding systems including monogamous and polygamous species mixed with biparental or uniparental care by the male and/or the female, and this diversity induced much research (Darwin 1871, Pitelka et al. 1974, Thomas et al. 2007). For example, there are polygamous species such as the ruff, *Philomachus pugnax*, a lek species in which males are larger than females and compete to mate with as many females as possible (Jukema & Piersma 2006). The reverse is also the case, in the polyandrous wattled jacana, *Jacana jacana*, females are larger and more aggressive than males. Males take care of the clutch and young for their own (Emlen & Wrege 2004). There is also partial male and female care such as in the sanderling, *Calidris alba* (Reneerkens et al. 2011), and the Kentish plover, *Charadrius alexandrinus* (Székely & Lessells 1993); and species with complete biparental care such as the Malaysian plover, *Charadrius peronii* (Yasué & Dearden 2007). Therefore, shorebirds are excellent model species to study the ecology and evolution of the breeding systems because they have unusual reproductive patterns (non-conventional sex roles behaviour in parental care and mating patterns), that allows to test current models of breeding systems evolution.

In this dissertation, I investigated the breeding ecology of small shorebirds from Madagascar. Specifically, plover species included in the genus *Charadrius* spp., which exhibit substantial variation in their breeding systems (Székely et al. 2006, Thomas et al. 2007, Zefania et al. 2010). There are four species of small plovers that co-occur in sympatry and breed in the wetland systems of the southwest coast of Madagascar: Kittlitz's plover, *Charadrius pecuarius*; Madagascar plover, *Charadrius thoracicus*; three-banded plover, *Charadrius tricollaris*; and white-fronted plover, *Charadrius marginatus* (Figure 1). These species are an excellent model system for studying their breeding behaviour for the following reasons. First, they are phylogenetically closely-related plover species that breed in sympatry (Dos Remedios 2014). Second, they exhibit similar life-histories and ecological traits (Appendix: Table 1). For example, they are small insectivorous ground-nesting birds nesting close to bodies of water. Third, Malagasy plovers are common breeding

species in much of the coasts of Madagascar and sub-saharan Africa (Urban et al. 1986; Hockey et al. 2005); although, the three-banded plover is found in low density where I carried out the fieldwork. Fourth, small plovers are good model species for experimental manipulations in the wild as they are amenable for experimentation (Székely & Cuthill 2000); for example, the manipulated individuals are straightforward to follow in their natural habitat. Fifth, the different parental care systems of the Malagasy plovers provide a great opportunity to assess theoretical expectations (Urban et al. 1986; Hockey et al. 2005). Finally, the breeding ecology of shorebirds has been poorly studied for tropical and subtropical species in contrast to northern species (Thomas et al. 2003).



**Figure 2.** A conceptual framework for breeding system evolution in relation to the Chapters of my dissertation. The framework shows feedback relationships between mating strategies, mating opportunities and parental care strategies and the resulting outcome of mating and parental care patterns (adapted from Székely *et al.* 2000).

### **Thesis outline**

Breeding system evolution has been linked to variation in the social environment (Jennions & Kokko 2010). This variation is exemplified by mating opportunities which are the result of different mating and parental care strategies in animals. The conceptual framework outlined in Figure 2 details important elements of breeding system evolution and their relationships. I use this framework to understand the interaction of social environment (mating opportunities) and observed mating and parental care behaviours of three closely-related Malagasy plover species: Madagascar plover (*Charadrius thoracicus*), white-fronted plover (*C. marginatus*) and Kittlitz's plover (*C. pecuarius*).

In this dissertation, I address a central question of breeding systems evolution which is: do mating opportunities shape animals breeding systems? I specifically asked whether mating opportunity of males and females is related to mating strategies, parental care strategies and spatial and temporal distribution of resources. The dissertation, therefore, is organized into six chapters describing different aspects of breeding systems by using experiments and natural behaviours of three Malagasy plovers. In chapter two, I focus on brood attendance behaviour of three species of plovers (Figure 2, parental care strategies). I tested whether brood attendance patterns differ between sympatric species of plovers. In chapter three, I experimentally assessed mating opportunities in three plover species which have different parental care patterns (Figure 2, mating opportunities). In addition, I evaluated courtship behaviour (Figure 2, mating strategies) and breeding vacancies in two Malagasy plovers (Figure 2, spatial and temporal distribution of resources). In chapter four, I investigated whether mate searching behaviour, spatial movements and social encounters are related with mating opportunities of the Kittlitz's and white-fronted plovers (Figure 2, spatial and temporal distribution of resources). In chapter five, I tested whether detectability differs between the sexes of young Malagasy plover (Figure 2, predation). In Chapter six, I summarised some important aspects studied of the breeding system of three Malagasy plovers. Finally, I discussed the overall conclusions of my dissertation and its contribution to our understanding of the complex relationships between mating patterns, mating opportunities and parental care patterns.

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## Appendix

**Table 1.** Aspects of breeding ecology of four Malagasy plover species (based on Zefania & Székely 2013). Means  $\pm$  SD are provided, unless stated otherwise.

Breeding ecology	Kittlitz's plover	Madagascar plover	Three-banded plover	White-fronted plover
Breeding habitat	mudflats, intertidal mud and short coastal grassland	mudflats, intertidal mud, sandy beaches, mangroves and short coastal grassland	mud edges and mudflats	sandy shores, rocky shores and mudflats
Breeding behaviour	Gregarious; breeding pair roots and foraging in groups; small territory during incubation	Territorial breeding pair	Territorial breeding pair	Territorial breeding pair
Clutch size	1.86 eggs range: 1 – 2	1.84 eggs range: 1 – 2	1.43 eggs range: 1 – 2	1.91 eggs range: 1 – 3
Incubation	27 - 28 days	26 - 28 days	29 - 33 days	26 - 29 days
Tarsus length at hatching	20.03 $\pm$ 0.76 mm	21.29 $\pm$ 0.61 mm	?	18.37 $\pm$ 0.71 mm
Weight at hatching	5.69 $\pm$ 0.5 g	7 g	4.9 g	5.89 $\pm$ 0.46 g
Adult tarsus (female)	31.67 $\pm$ 1.36 mm	32.84 $\pm$ 1.06 mm	26.93 $\pm$ 1.78 mm	25.65 $\pm$ 1.31 mm
Adult weight (female)	35.59 $\pm$ 2.79 g	37.68 $\pm$ 2.68 g	40.25 $\pm$ 4.60 g	37.33 $\pm$ 2.12 g
Adult tarsus (male)	31.73 $\pm$ 1.36 mm	33.34 $\pm$ 1.11 mm	26.67 $\pm$ 0.85 mm	25.94 $\pm$ 0.86 mm
Adult weight (male)	34.42 $\pm$ 2.4 g	36.45 $\pm$ 2.22 g	35.13 $\pm$ 1.7 mm	36.69 $\pm$ 2.54 g

## CHAPTER TWO

### **Brood Attendance Behaviour in Three Malagasy Plovers**

Jorge E. Parra, Marcela Beltrán, Sama Zefania, Natalie  
Dos Remedios and Tamás Székely

*Manuscript*

*Author's contributions*

**JEP:** study design, data collection, molecular analysis, statistical analysis,  
manuscript preparation

**MB:** data collection in the field

**SZ:** data collection in the field

**NDR:** molecular analysis, manuscript improvement

**TSz:** study design, manuscript improvement

### **Abstract**

Parental care is costly and parental care strategies are dependent on the trade-off between the benefits of care provisioning and the benefits of deserting the young and remating. This trade-off may be resolved in different ways for different species, even those that are ecologically similar. However, evidence of variation in parental care is scant in tropical and subtropical populations. Here we compare brood care behaviour between three plover species that breed in the same habitat in subtropical Madagascar. Families of the Madagascar plover (*Charadrius thoracicus*), white-fronted plover (*C. marginatus*) and Kittlitz's plover (*C. pecuarius*) were colour ringed and parental brood attendance behaviour was observed across multiple years. We found that brood attendance behaviour is highly variable between the three species of plover. Male-only and biparental brood attendance was more common than female-only brood care in the Madagascar and the white-fronted plovers. In Kittlitz's plover, brood attendance was male-biased in all chick age categories. For all three species, males seemed to play an important role in attending broods whereas female plovers tended to reduce or share their care roles. Results suggest that parental care behaviours are influenced by the species of plover and the age of chicks. Our study demonstrates that parental care is variable between sympatric and closely related plover species. We argue that interspecific variation in brood care is influenced by mate availability and breeding vacancies, which in turn influence the trade-off between the benefits of caring and the benefits of deserting.

**Keywords:** brood attendance, Malagasy plovers, mating systems, parental care, precocial.

## Introduction

Parental care is any form of behaviour by a parent that increases the survivorship and reproductive success of their offspring (Smiseth *et al.* 2012). These behaviours involve expenditure of time and energy, such that investment by parents in their current offspring diminishes their ability to invest in other current or future offspring (Trivers 1972). Parents provide care in form of nourishment, protection against predators and parasites, food provisioning, attendance of eggs and brood attendance, among other elaborate forms of care (Clutton-Brock 1991). This provision of care is highly variable between different animal taxa in terms of duration, type of care and the sex of the care provisioning parent. For example, in 90% of mammal species, females provide the majority of care in line with physiological constraints on males (e.g. gestation, lactation; but see Kunz & Hosken 2009). However, among other taxa, male care is more common and male investment occurs in two forms: care provided by both the male and female (biparental care) such as in 90% of bird species, some frog and primate species (Cockburn 2006, Reynolds *et al.* 2002); and male-only care, such as in most of teleost fishes, anurans and some species of birds (Reynolds *et al.* 2002; Summers *et al.* 2006). Parental care patterns can be highly variable even within populations of the same species in birds and fishes (Persson & Öhrström 1989; Davies & Lundberg 1984; Székely & Cuthill 1999; Vincze *et al.* 2013).

Explaining the evolution and diversity of parental care patterns has been challenging. Theoretical work suggests that an important component in the evolution of parental care patterns is the trade-off between the benefits of caring for, and the benefits of deserting, the offspring (Maynard-Smith 1977; Clutton-Brock 1991; Klug *et al.* 2012). On the one hand, care provides benefits by increasing the fitness of parents' offspring. This is achieved through behaviours such as building nests, attending eggs, brooding offspring, providing food and even caring for mature offspring. On the other hand, desertion is beneficial to a parent if this increases its survival for future reproductive attempts, or if there are additional mating opportunities over the breeding season. Therefore, a parent is expected to care for its offspring if the benefits of care exceed the benefits of desertion (Reynolds 1996; Székely *et al.* 2000). Since parental investment improves the fitness of the offspring at a cost to the future fitness of the investing parent (Trivers 1972; Maynard-Smith 1977), a conflict of interest between males and females emerges and each parent

might prefer that the other makes up the bulk of parental investment (Houston et al. 2005; Székely et al. 2007). The resolution of this sexual conflict involves different modes of manipulation between sexes including exploiting signalling systems, coercion over parental investment and deception (Arnqvist & Rowe 2005).

Shorebirds (plovers, sandpipers and allies) are an excellent group for investigating parental care for three reasons. First, they are a diverse avian order (*Charadriiformes*) distributed on every continent, breeding on coastal and inland habitats. Second, they have a remarkable diversity of breeding ecology and life history traits (e.g. migratory behaviours, Garcia-Pena *et al.* 2009). Finally, shorebirds exhibit highly variable breeding systems, and mating systems in most cases match with parental care patterns. Some species are monogamous and both parents rear the young, whereas others exhibit polygyny and/ or polyandry and a single parent raises the young (Reynolds & Székely 1997; Székely et al. 2006; Thomas et al. 2007).

The breeding ecology of shorebirds has been relatively well studied for northern species in contrast to tropical and subtropical species (Thomas *et al.* 2003). Some studies in tropical and southern hemisphere birds have shown substantial differences in avian life-history traits comparing with north temperate birds. For example, higher adult survival was observed in tropical shorebird species than in temperate shorebird species (Lloyd 2008). In addition, southern birds in general have shown higher rates of nest predation, extended and / or more variable reproductive periods, more nesting attempts per year and more extended parental care and monogamy than northern birds (Martin *et al.* 2000). These and other factors might cause, in turn, major differences in social behaviours that have not yet been exhaustively studied.

In this study, we investigated sex roles during parental care among three shorebird species. Specifically, we studied the brood attendance behaviour by males and females in three closely-related plover species that breed in sympatry in subtropical Madagascar (dos Remedios 2014). Brood attendance is a form of parental care in which the parent(s) of precocial young provide them with insulation to aid thermoregulation and also guard them to provide protection from danger. This parental care behaviour has been studied in all three species: the Madagascar plover,

*Charadrius thoracicus*, an endemic species to Madagascar usually found in pairs throughout the breeding period (Urban *et al.* 1986); the white-fronted plover, *C. marginatus*, which exhibits biparental care in African populations (Urban *et al.* 1986; Hockey *et al.* 2005); and the Kittlitz's plover, *C. pecuarius*, which reportedly exhibits biparental care as well as uniparental care by either the male or female parent (Tree 1974; Urban *et al.* 1986). Brood care has not been investigated thoroughly for any of these species, and only anecdotal information are available. This is in contrast with brood care patterns studied in closely-related plover species that breed in the northern hemisphere such as the ringed plover *Charadrius hiaticula* (Wallander & Andersson 2003), the mountain plover *Charadrius montanus* (Graul 1975; Dreitz 2009) and the Kentish plover *Charadrius alexandrinus* (Kosztolanyi *et al.* 2006), among others (Thomas *et al.* 2003).

Using data from four different years, the objectives of this study were: (i) to compare whether males and females of the three plover species differ in their brood attendance behaviour and (ii) to investigate whether the age or hatch date of broods may influence brood attendance behaviour.

## Methods

### *Study sites and target species*

Fieldwork was carried out between December and June in 2005, 2010, 2011 and 2012, focused on three areas in southwest Madagascar: Andavadoaka (22° 02' S, 43° 39' E), Toliara (23° 24' S, 43° 43' E) and Tsimanampetsotsa National Park (24° 3' S, 43° 44' E). In these sites, all three plover species breed on mud-banks, sand-banks and short coastal grassland around saltmarshes and beaches. To capture plovers, nests were searched for every day using a mobile hide and car around the breeding sites (Székely *et al.* 2003). Parents were captured by placing a funnel trap on the nest or over downy chicks. Adults and chicks were ringed with an individual combination of colour and numbered SAFRING metal bands from the University of Cape Town, South Africa.

### *Molecular sexing*

In order to determine the sex of adult plovers, a small blood sample was taken from the leg vein and brachial wing vein by puncturing, collecting drops of blood (~25 ul)

in capillary tubes, and storing the blood in Eppendorf tubes of Queen's Lysis Buffer for molecular sex-typing (see details in Kentish plover field guide, [www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/KP\\_Field\\_Guide\\_v3.pdf](http://www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/KP_Field_Guide_v3.pdf)). Molecular sex-typing was carried out in the NERC-Biomolecular Analysis Facility at the University of Sheffield.

DNA was extracted from blood samples using the Ammonium Acetate extraction method (Miller *et al.* 1988; Richardson *et al.* 2001). For molecular sex-typing, Z- and W-chromosome specific genes were amplified via the Polymerase chain reaction using the Z-002B/Z-002D primers (Dawson 2007). For additional certainty in sex assignment, the W-chromosome specific Calnex-31 primers, developed in the genus *Charadrius* were utilized (Küpper *et al.* 2006). PCR amplification was conducted on a DNA Engine Tetrad 2 Peltier Thermal Cycler under the following conditions: 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 56°C for 90 s, 72°C for 60 s with a final extension of 60°C for 30 min. Samples were visualized on an ABI 3730 automated sequencer. IR Dye-labeled tailed primers separated the product of Z-002B/Z-002D primers into either one (ZZ) or two bands (ZW), indicating male or female respectively. The W-specific Calnex-31 product appeared as one band indicating female only. Images were scored using GeneMapper software version 4.1 (Applied Biosystems). To maximize reliability, all samples were sexed using two markers. Additionally, for 8% of samples (11 Kittlitz's plover and 10 White-fronted plover individuals) molecular sexing was repeated, with 100% concurrent results.

### *Plover families and behaviour*

Plover chicks captured on the day of hatching were ringed, monitored, recaptured and tarsus length was repeatedly measured until fledging (age 25 days). Since tarsus growth is linear with age among Kentish plovers (Székely & Cuthill 1999), these measurements were used to estimate the age of chicks that were only encountered after leaving the nest (see statistical methods).

The attending parent(s) and brood size were determined by observing families of each species from a mobile hide or car for at least half an hour. This period was sufficient to record whether both parents were guarding the chicks (Székely & Cuthill 2000). Each family was subsequently classified into one of three



categories: ‘male-only’, ‘female-only’ and ‘biparental’ brood attendance (Table 1). Colour rings were used to identify the members of broods. Molecular sexing was sufficient to allow the unequivocal identification of the sex of breeding adults. Finally, each family was included in a chick age category (<2, 3-7, 8-16, >17 days) following the developmental pattern from downy to juvenile plumage (Table 1).

### *Statistical analyses*

Linear growth equations were used to estimate the age of chicks based on measurements from 33 Madagascar plovers (69 measurements), 13 white-fronted plovers (28 measurements) and 28 Kittlitz’s plovers (54 measurements). For Madagascar plovers: age (days) =  $2.43 * \text{tarsus (mm)} - 50.99$ ,  $r^2 = 0.911$ ; for white-fronted plovers: age (days) =  $2.804 * \text{tarsus (mm)} - 50.52$ ,  $r^2 = 0.920$ ; and for Kittlitz’s plovers: age (days) =  $2.84 * \text{tarsus (mm)} - 55.23$ ,  $r^2 = 0.927$ .

Brood age was the sum of all chick age values in a brood divided by brood size (number of chicks). For each brood, hatching date was also estimated using the brood encounter date and brood age when hatching date was not known. Brood age and hatching date variables were centred by subtracting the median value of all broods from the values of each individual brood.

We used a generalized linear mixed model (GLMM) with multinomial probability distribution and cumulative logit link function to evaluate the probability of brood attendance behaviour (male-only, female-only and biparental care) as a function of plover species (Kittlitz’s, Madagascar and white-fronted plovers), year of study (2005, 2010, 2011 and 2012), brood age, hatching date and second order interactions of these variables. Few broods held more than one attendance behaviour measurements resulting in nonindependence of the data. We fitted individual brood identity as the random term in the model to account for repeated measures of different broods within the same species and year. The model was simplified by removing nonsignificant interactions ( $P > 0.05$ , reverse-stepwise). The fitted model included only fixed terms and significant second order interactions. Statistical tests were two tailed with  $\alpha = 0.05$ . GLMM were calculated using IBM SPSS Statistics 20 (IBM Corp.). In addition, predicted probabilities of brood attendance were plot against plover species and chick age by species using the package ‘ggplot2’ (Wickham 2009) for R (R Core Team 2010).

## CHAPTER TWO

**Table 1.** Variables used to analyze brood attendance behaviour in three species of Malagasy plovers *Charadrius spp.* Table shows the mean and standard deviations.

Species		age of chicks (days)			
		0-2	3-7	8-16	17+
Madagascar plover	brood size	1.79 ± 0.41	1.57 ± 0.51	2.0 ± 0.00	1.76 ± 0.44
	age of chicks	2.17 ± 0.51	4.56 ± 1.64	13.43 ± 4.26	20.90 ± 4.12
	number of observations	24	23	3	25
	number of families	7	8	2	7
white-fronted plover	brood size	1.06 ± 0.25	1.67 ± 0.52	1.52 ± 0.59	1.21 ± 0.41
	age of chicks	1.63 ± 1.10	5.25 ± 1.53	12.12 ± 2.80	23.60 ± 3.81
	number of observations	31	48	25	75
	number of families	13	17	13	24
Kittlitz's plover	brood size	1.13 ± 0.34	1.44 ± 0.5	1.26 ± 0.44	1.40 ± 0.49
	age of chicks	1.13 ± 1.22	4.89 ± 1.52	12.32 ± 2.72	24.78 ± 4.86
	number of observations	62	63	72	58
	number of families	22	23	23	21

## Results

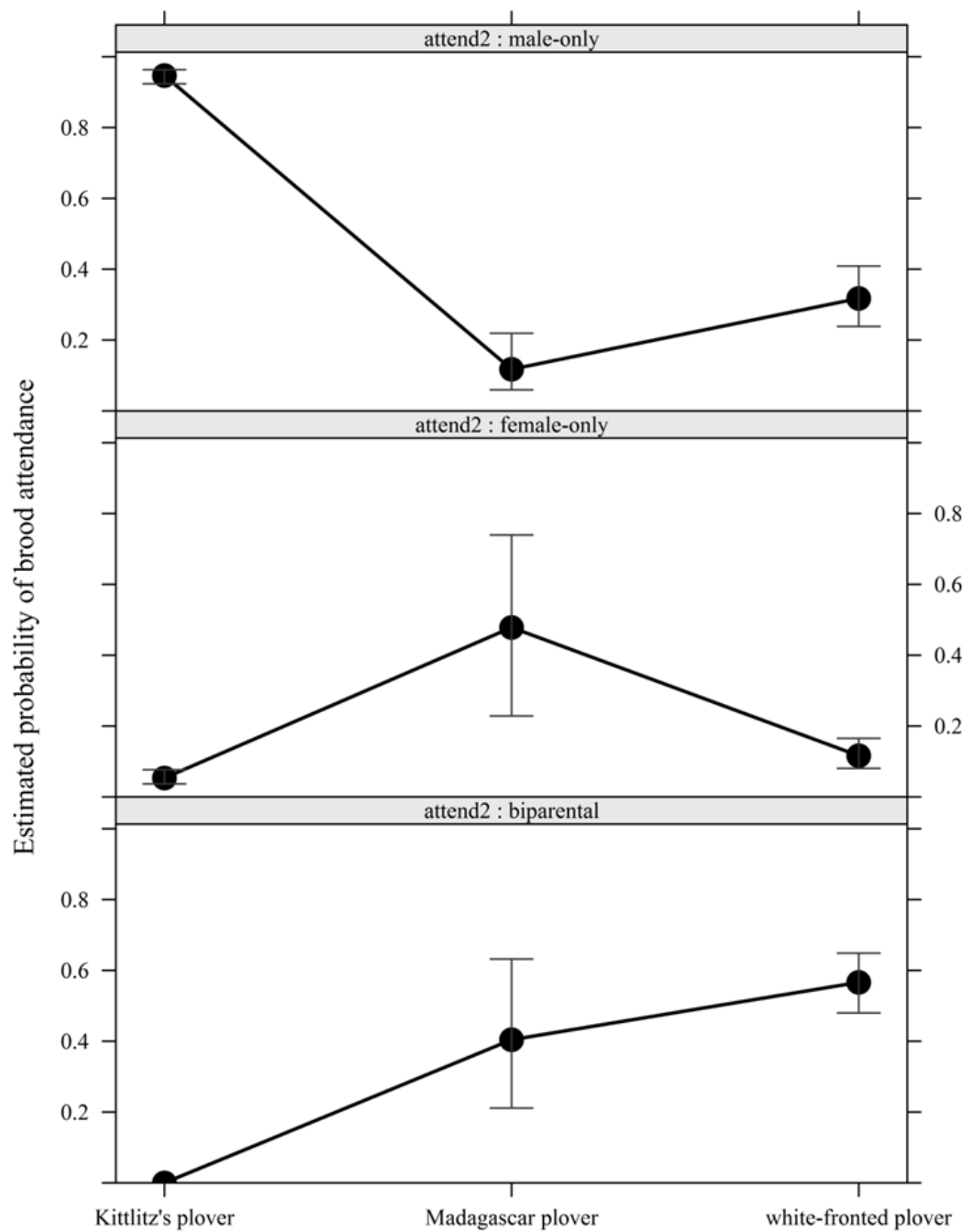
Brood attendance behaviours were different between Malagasy plovers (Table 2). On the one hand, brood attendance was provided for both males and females in Madagascar and white-fronted plovers (Figure 1). On the other hand, male Kittlitz's plovers significantly provided most of the care to chick (Figure 1). For all species, females tended to provide less care than males; females were more often engaged in biparental care than female-only care (Figure 1).

The interaction between plover species and brood age had significant effects on brood attendance patterns (Table 2). Brood attendance behaviour depends on brood age in Madagascar and white-fronted plovers. As chick age, biparental brood care increase and male-only care decrease in the Madagascar plover (Figure 2); and male-only care increase and biparental care decrease in the white-fronted plover (Figure 2). In contrast, male-only care remains constant as chick as age whereas female-only and biparental care seems no to play an important role in brood attendance for the Kittlitz's plover (Table 2, Figure 2).

**Table 2.** Estimated effect sizes  $\pm$  SE (standard errors), t values and p values for fixed effects in GLMM with multinomial probability distribution (male-only, female-only and biparental care) and cumulative logit link function. Reference category: biparental care.

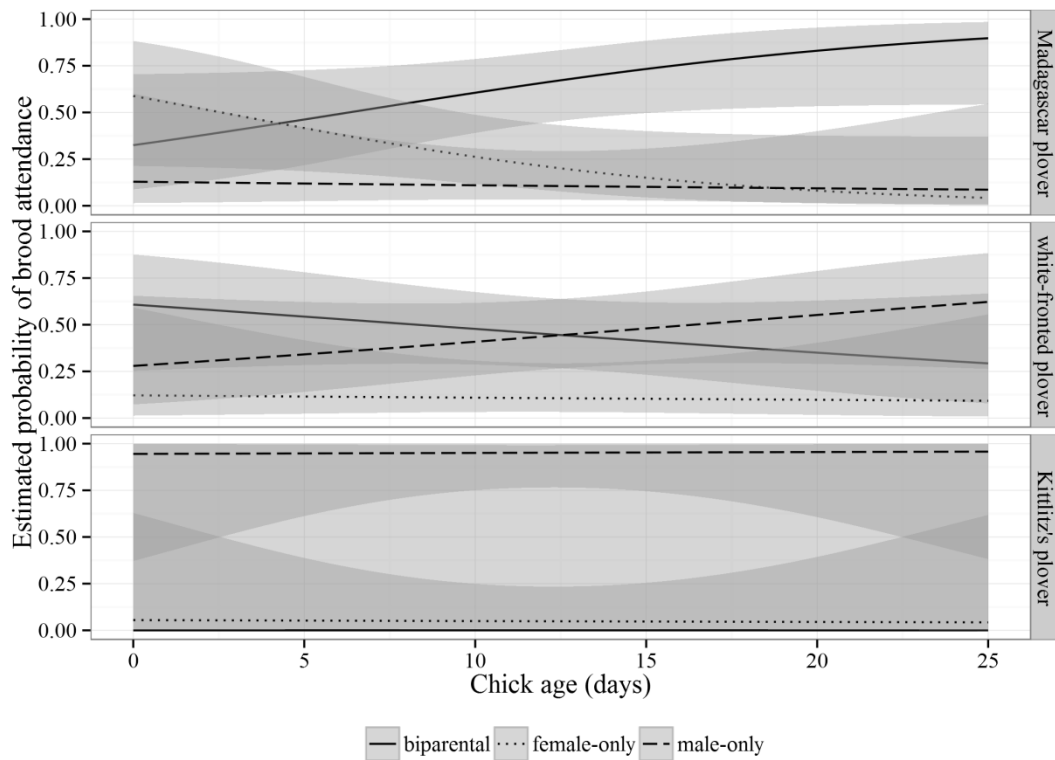
Model terms	coefficient $\pm$ SE	t-test	P
Species			
Kittlitz plover (Kip)	-5.64 $\pm$ 2.38	-2.38	<b>0.018</b>
Madagascar plover (Mp)	-2.51 $\pm$ 2.75	-0.91	0.362
white-fronted plover (Wfp) <sup>b</sup>	0 <sup>a</sup>	-	-
Year			
2005	0 <sup>a</sup>	-	-
2010	0.48 $\pm$ 2.66	0.18	0.857
2011	-1.38 $\pm$ 2.02	-0.69	0.494
2012 <sup>b</sup>	0 <sup>a</sup>	-	-
Brood age	-0.07 $\pm$ 0.03	-2.18	<b>0.030</b>
Hatching date	-0.01 $\pm$ 0.01	-1.03	0.303
Species x brood age			
Kip x brood age	0.04 $\pm$ 0.05	0.74	0.459
Mp x brood age	0.15 $\pm$ 0.07	2.17	<b>0.030</b>
Wfp x brood age <sup>b</sup>	0 <sup>a</sup>	-	-

<sup>a</sup> This coefficient is set to zero because it is redundant. <sup>b</sup> Reference value for species, year and species x brood age.



**Figure 1.** Predicted probabilities of brood attendance as a function of plover species. Data presented are means and standard errors calculated from the GLMM.

## PARENTAL CARE



**Figure 2.** Predicted probabilities of brood attendance as a function of chick age for three species of Malagasy plovers obtained by GLMM. Lines include shaded standard errors.

## Discussion

Using the most detailed data yet available, we showed that brood care patterns differed significantly between plover species. First, we found that male plovers played an important role in providing brood attendance whereas female plovers tended to exhibit a lower level of care or share brood attendance with their mate (Fig. 1 and 2). This pattern is consistent with studies conducted among some north temperate species of shorebirds, for example *Calidris spp*, where females share incubation but exhibit reduced brood attendance relative to males. This may be explained by the high brooding requirements of younger chicks in comparison with older chicks. For example, biparental brood attendance might be needed to keep downy chicks warm and/or protect them in areas with high rates of predation (Miller 1985). Secondly, brood care patterns varied according to chick age in the socially monogamous Madagascar and white-fronted plovers. The variable brood care behaviour exhibited by males and females is probably due to differences in the

defence of territorial resources by the pair over the season. In some birds, for example, females tended to be more aggressive against rival females to guard and retain the parental investment of their mates and territorial resources (Yasukawa & Searcy 1982). Finally, male-only brood care was the most common behaviour observed in the Kittlitz's plover during offspring development. In this species, brood care has been reported as biparental and female-biased until fledging in South Africa (Tree 1974), and male-biased in captivity (Urban *et al.* 1986). However, the South African study did not use individual marking and the report on captive Kittlitz's plover referred to one family only (Tree 1974; Urban *et al.* 1986; Hockey *et al.* 2005).

The variable brood attendance patterns observed among Malagasy plovers is consistent with the diversity of breeding systems reported across shorebird species with precocial young. First, it has been argued that less demanding young open up the possibility for a diversity of parental care strategies (Thomas & Székely 2005; Székely *et al.* 2007). For instance, in the Kentish plover, *Charadrius alexandrinus*, no difference in chick survival and growth was found between broods with uniparental care (male or female-only) or biparental care (Székely & Cuthill 1999). This suggests that one parent alone can successfully rear precocial young to independence under favourable ecological conditions.

Secondly, resource variation may influence parental care patterns. For instance, when resources are plentiful, one sex might be more likely to desert the offspring, leaving the other sex to carry out parental care duties (Emlen & Oring 1977). Brood care strategies are highly variable within populations of the Kentish plover, *C. alexandrinus*, where variation in food abundance and intraspecific competition are likely to have constrained females to desert the brood rather than males (Kosztolanyi *et al.* 2006). Female-biased nest desertion and male-only care were positively associated with food abundance in the Tengmalm's owl, *Aegolius funereus* (Eldegard & Sonnerud 2009). In contrast, biparental care strategies were exhibited to compensate for losses and maximise offspring survival when resource distributions were patchy (e.g. breeding and feeding territories) in the oystercatcher, *Haematopus ostralegus* (Heg & van der Velde 2001). Two of our study plover species, the Madagascar plover and the white-fronted plover, present territorial behaviour against conspecifics and other shorebirds during periods of brood care

attendance (Urban *et al.* 1986; Hockey *et al.* 2005). This suggests that biparental brood attendance behaviour might involve not only attending mobile young but also defending scarce territorial food resources. Pairs of white-fronted plovers, for instance, feed and breed exclusively on sandy intertidal coasts. In this species, territories seem to be important resources as pairs exhibited high mate and site fidelity within and between years in South Africa (Lloyd 2008). In contrast, Kittlitz's plover families (adult male and chick(s)) left their nesting areas and moved towards feeding sites along shorelines, grass lands and salt pans (personal obs.). In this species, uniparental brood attendance is probably enough because broods join groups of plovers on shores with high food abundance. In addition, having several plover broods in a group may decrease the probability of predation (van den Hout *et al.* 2008).

Third, the ratio of sexually active males to females (the operational sex ratio – OSR (Emlen & Oring 1977; Kvarnemo & Ahnesjö 1996)) has been proposed to influence future mating opportunities and parental care behaviour. Thus, with a male-biased OSR, theoretical models predict that males should either intensify competition for the scarce females (Trivers 1972) or there may be selection for increased male care (McNamara *et al.* 2000, Kokko and Jennions 2008). In a previous study, we found that mating opportunities were not biased in the biparental White-fronted plover, and were male-biased in the uniparental Kittlitz's plovers in Madagascar (Parra *et al.* 2014). We infer from this evidence that in the white-fronted plover, biparental care behaviour is probably limited by ecological resources other than mates (e.g. nesting sites), as males and females had equivalent mating opportunities. In the Kittlitz's plover, male-only brood attendance most likely creates a female-biased OSR, there are probably more sexually-active females than males; increasing male mating opportunities at the same time. This study cannot test this, without estimates of plover adult sex ratios.

Our results showed two different male brood care patterns in the three species. Firstly, males shared brood attendance with females and took over parental care during the late pre-fledging period of chicks. This pattern might emerge due to limited resources such as food, breeding vacancies and mates, at which time both sexes are needed to defend scarce territories and to rear the offspring at the same time. Secondly, male-only brood care was the most common pattern exhibited by the

Kittlitz's plover. Male-only parental care behaviour probably emerges due to either low mating opportunities for males or high male-male competition. In addition, the Kittlitz's plover's variable breeding system is probably related to their complex social behaviours. For example, pair-bond formation occurs in gregarious groups prior to territory acquisition, males defend nest-sites, breeding parents feed in gregarious groups and territory dilution occurs soon after chicks hatch (Urban *et al.* 1986; Hockey *et al.* 2005). Taken together, these results are striking because Madagascar plover and Kittlitz's plover are phylogenetically more closely related to each other than to the White-fronted plover (dos Remedios 2014) however, they differ greatly in their parental care behaviours. Therefore, ecological and social factors seem to be important in determining how breeding systems evolve.

Here, we investigated the variable brood attendance behaviour of three Malagasy plovers breeding in the southwest Madagascar. The Madagascar plover showed a similar male-only and biparental brood attendance in all chick age categories. The White-fronted plover exhibited biparental brood attendance behaviour. Finally, the Kittlitz's plover presented consistent male-biased brood attendance between years. Our study demonstrates that parental care is highly variable between sympatric and closely-related plover species and shows that shorebirds are excellent species for studying the evolution of breeding systems. Further work will be required to understand how ecological constraints and population dynamics influence the cost-benefit ratio associated with the parental care behaviour observed in these Malagasy plover populations.

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## CHAPTER THREE

### **Experimental assessment of mating opportunities in three shorebird species**

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*Author's contributions*

**JEP:** study design, data collection, molecular analysis, statistical analysis, manuscript preparation

**MB:** data collection in the field

**SZ:** data collection in the field

**NDR:** molecular analysis, manuscript improvement

**TSz:** study design, manuscript improvement

## Abstract

Mating opportunities may differ between closely related species, although the evidence for such variation is scant. Here we compared remating opportunities and courtship behaviour between three shorebird species: the Kentish plover, *Charadrius alexandrinus*, the Kittlitz's plover, *Charadrius pecuarius*, and the white-fronted plover, *Charadrius marginatus*, using data and an experimental approach from a previous study of the Kentish plover. By experimentally creating unmated males and females, we found that remating opportunities differed between these closely related plover species: remating times were shorter for females than males in a Kentish plover population that had a male-biased adult sex ratio, and in which most brood care after hatching was carried out by males. In contrast, remating times were male-biased in the uniparental Kittlitz's plover and unbiased in the biparental white-fronted plover. Male Kentish plovers spent significantly more time on courtship than females, whereas courtship behaviour was not sex biased in the other two plover species. The mate removal experiments also provided insights into pair bond stability. In the Kittlitz's plover, all 16 newly formed pairs remained together after the release of their former mates from captivity, whereas newly established pairs were replaced by their former mates upon release in all 12 white-fronted plover pairs. Taken together, these results are important in highlighting interspecific variation in mating activities, and suggest that both operational sex ratio and pair bond stability may differ between closely related species. These variations, in turn, may influence mating systems and parental care.

**Keywords:** adult sex ratio, mating system, operational sex ratio, pair bond, parental care, remating opportunity.

## Introduction

The different evolutionary interests of males and females over reproduction (termed sexual conflict; Parker, 1979) are a pervasive evolutionary force influencing the behaviour, ecology and life histories of many organisms (Arnqvist & Rowe, 2005; Chapman, Arnqvist, Bangham, & Rowe, 2003). A common issue when the interests of males and females are antagonistic concerns offspring care (Trivers, 1972; Maynard Smith, 1977; Houston, Székely, & McNamara, 2005; Lessells, 2012). By caring for the offspring, parents often improve the growth and survival of the young (Clutton-Brock, 1991; Klug, Alonzo, & Bonsall, 2012); for example, by gestating, nursing, protecting and feeding them, parents improve the offspring's chances of survival (Tyler, Shearman, Franco, O'Brien, Seamark, & Kelly, 1983; Balshine-Earn & Earn 1998; Baeza & Fernández, 2002; Klug, Alonzo, & Bonsall, 2012). However, care is costly in terms of time and energy, and the caring parent can be killed by predators or may lose additional mating opportunities (Veasey, Houston, & Metcalfe, 2001; Li & Jackson, 2003; Klug et al., 2012). Therefore, although both biological parents benefit from providing care for the offspring, each parent is expected to withhold his (or her) parental contribution in order to raise further offspring in future (Houston et al., 2005; Lessells, 2012; McGraw, Székely, & Young, 2010).

Theory suggests that a key component of conflict resolution between male and female parents is mating opportunity (Székely, Webb, & Cuthill, 2000; McNamara, Székely, Webb, & Houston, 2000; Kokko & Jennions, 2008; Klug et al. 2012). If one sex has more favourable mating opportunities than the other, this parent is expected to reduce (or completely terminate) care more often than its mate, and seek out a new partner (Balshine-Earn & Earn, 1998; Owens, 2002; Pilastro, Biddau, Marin, & Mingozi, 2001). One approach used by researchers to assess mating opportunities is to estimate the ratio of sexually active males to females (operational sex ratio, OSR; Kvarnemo & Ahnesjö, 1996; Forsgren, Amundsen, & Bjelvenmark, 2004). An alternative approach to estimating mating opportunities is to create unmated individuals experimentally, and to quantify their remating behaviour, for example time to remate, remating success and reproductive success with the new mate (Lessells, 1983; Székely, Cuthill, & Kis, 1999). This experimental approach is



powerful, since it directly assesses the mating potential of unmated individuals at a given time in a population.

In this study, we estimated remating opportunities for two small plover species, the white-fronted plover, *Charadrius marginatus*, and the Kittlitz's plover, *Charadrius pecuarius*, and we compared these data with the results of a previous study on the Kentish plover, *Charadrius alexandrinus* (Székely et al., 1999). Small plovers (*Charadrius* spp.) exhibit substantial variation in their breeding systems, since some of these species are monogamous and both parents rear the young, whereas others exhibit polygyny and/or polyandry whereby a single parent (the male or the female) raises the young to independence (Székely, Thomas, & Cuthill, 2006; Thomas, Székely, & Reynolds, 2007). In addition, plovers typically breed in open areas, and their nests and broods are therefore accessible for experimental manipulations (Székely & Cuthill, 2000).

A previous experiment established that remating opportunities were female-biased in the Kentish plover (Székely et al., 1999), and this result was consistent with demographic analyses that estimated about six times more adult males than females in the population (Kosztolányi, Barta, Küpper, & Székely, 2011). Skewed adult sex ratios (ASRs) are common in wild populations (Donald, 2007), and recent studies suggest that biased ASRs predict sex roles, mating systems and pair bonds (Liker, Freckleton, & Székely 2013). We used an identical experimental protocol with two close relatives of the Kentish plover, the white-fronted plover and Kittlitz's plover, to compare remating opportunities between these three plover species. All three species are insectivorous ground-nesting birds with similar life histories and ecology (adult body mass: Kentish plover: 41.8 g; white-fronted plover: 37.1 g; Kittlitz's plover: 35.3 g, Urban, Fry, & Keith, 1986; Hockey, Dean, & Ryan, 2005). The latter two species are common breeding birds in Africa, and their parental care systems differ from that of the Kentish plover which exhibits male-biased parental care after hatching (Lessells, 1984; Székely & Lessells, 1993; Amat, Fraga, & Arroyo, 1999). White-fronted plovers exhibit biparental brood care, whereas Kittlitz's plovers are reported to exhibit uniparental brood care, carried out by either the male or the female parent (Hockey et al., 2005; Tree, 1974; Urban et al., 1986).

Based on theoretical models (Klug et al., 2012; Kokko & Jennions, 2008) and available information on patterns of parental care (Hockey et al., 2005; Urban et al., 1986), we derived three predictions. First, we predicted higher remating opportunities in uniparental species (Kittlitz's plover) than in biparental species (white-fronted plover), since in biparental species both parents are engaged with care until the offspring are fully independent (henceforth, between-species comparison). Second, we predicted no difference in remating opportunities between males and females in biparental white-fronted plovers given that both sexes are fully engaged in parental care. Similarly, no difference in remating opportunities between males and females was predicted for the uniparental Kittlitz's plover in which either parent is free to seek a new mate (henceforth, between-sexes comparison). Third, we predicted intense courtship behaviour by males and females in both biparental white-fronted plovers and uniparental Kittlitz's plovers where care is provided by either parent (henceforth, courtship behaviour). In addition to the experimental assessment of remating opportunities, we also monitored pair bond stability among newly established pairs. We include the Kentish plover in our analyses (using the data from Székely et al., 1999), since the same experimental methodology was used in all three species. Nevertheless, our main conclusions remain consistent when restricting the analyses to the white-fronted and Kittlitz's plovers.

## Methods

### *Study Species and Study Sites*

White-fronted plovers and Kittlitz's plovers were investigated in southwest Madagascar (for Kentish plover, see details in Székely et al., 1999). Kittlitz's plovers were studied between 6 February 2010 and 13 May 2010 in Andavadoaka (22° 02'S, 43° 39'E) where they breed around alkaline lakes. Approximately 300 Kittlitz's plovers breed in Andavadoaka (J.E. Parra, S. Zefania, & T. Székely, unpublished data). Fieldwork with the white-fronted plover was carried out between 1 April 2011 and 23 June 2011 at Lake Tsimanampetsotsa National Park (24° 3'S, 43°44'E), a large alkaline lake (approximately 15 km x 0.5 km), surrounded by sandy shores, short grass and saltpans. Approximately 150 white-fronted plovers breed around the lake (J.E. Parra et al., unpublished data).

In the field, we searched for nests on foot, identified incubating parents and watched the parent(s) returning to nests in potential breeding sites. In total, we captured 18 Kittlitz's plover pairs (36 individuals) and 14 white-fronted plover pairs (28 individuals) with funnel traps placed on their nests. The traps were continuously monitored until a parent entered the trap and sat on the eggs. Parents were immediately removed from the traps to reduce stress and the risk of injury. Morphological traits (body mass, tarsus length, wing length and bill length) were measured using a spring balance, a sliding calliper and wing ruler (see details in Kentish plover field guide, [www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/KP\\_Field\\_Guide\\_v3.pdf](http://www.bath.ac.uk/bio-sci/biodiversity-lab/pdfs/KP_Field_Guide_v3.pdf)). All adults were ringed with an individual combination of colour rings and a numbered SAFRING metal ring from the University of Cape Town, South Africa.

#### *Experimental Manipulation*

We used the methodology developed by Székely et al. (1999) to estimate remating times in the Kentish plover. Briefly, both parents were trapped, ringed, measured and a blood sample was taken for sex determination (see below). One parent was then selected at random (the male or the female) and was released at the capture location immediately. The other parent was taken into captivity (see below). In both Kittlitz's and white-fronted plovers, both the male and female incubate the eggs (Hockey et al., 2005; Urban et al., 1986). Only pairs incubating two eggs (modal clutch size in both species) were manipulated. Egg length and breadth were measured with a sliding calliper, and the number of days for which the eggs had been incubated was estimated based on the floatation stage of the egg in a transparent jar with clean water (mean  $\pm$  SD number of days incubated: Kittlitz's plover:  $9.0 \pm 4.32$  days,  $N = 36$ ; white-fronted plover:  $11.5 \pm 3.16$  days,  $N = 20$ ). Eggs were distributed to other nonexperimental plover clutches at approximately the same stage of incubation in the local populations. Monitoring the augmented clutches was beyond the scope of the experiment, although casual nest checks suggest that at least 33.3% and 19.4% of augmented nests survived until hatching in the Kittlitz's plover ( $N = 36$  nests) and the white-fronted plover ( $N = 20$  nests), respectively. Survival in these nests appeared to be higher than for unmanipulated nests (13.4% and 8.9%, based on  $N = 101$  Kittlitz's plover nests and  $N = 56$  white-fronted plover nests, respectively; J.E. Parra et al., unpublished data).

Removed plovers were transported in an air-conditioned vehicle to a purpose-built aviary near the field camp at both study sites. Lightweight bird bags were used to keep the plovers undisturbed and ventilated during the transport. Distance from capture areas to the aviaries varied between 1 and 10 km in both study sites. The aviaries had four units for Kittlitz's plovers and six units for white-fronted plovers. Each unit consisted of a wood frame (1.5 x 1.5 m and 1 m high) fitted with chicken mesh (13 mm x 13 mm). To provide shade for the captive birds, we covered the outside of the aviary with papyrus, *Cyperius* sp., and fitted 50 cm of cloth at the base of the mesh inside the units. Captive plovers were provided with appropriate food and drink three times every day to maintain their good health using high-protein meals: dried invertebrates for wild birds (dried mealworms, dried earthworms, shrimps and dried waterfly; [shop.naturesgrub.co.uk/](http://shop.naturesgrub.co.uk/)), bird supplement vitamins (Vitacombex V; [www.petland.co.uk](http://www.petland.co.uk)) and pinhead oatmeal (Prosecto Insectivorous™; [www.haiths.com](http://www.haiths.com)). Captive plovers were also supplied with fresh insects twice a day using pit fall traps set up in the salt marsh. In addition, two water recipients were set up for drinking and bathing in each unit. Captive plovers were released after their former mate either found a new mate or was not seen in the study sites for at least 12 days. Time in captivity was comparable for white-fronted plovers (mean  $\pm$  SD number of days in captivity:  $8.0 \pm 1.71$  days,  $N = 14$ ) and Kittlitz's plovers ( $7.12 \pm 2.57$  days,  $N = 18$ ). Captive plovers were measured before release. Although captive plovers appeared to lose a small amount of body mass during their time in captivity ( $2.77 \pm 0.51$  g in Kittlitz's plover, and  $0.73 \pm 0.22$  g in white-fronted plover), they were in good condition as indicated by the fact that many remated shortly after release from captivity (see Results).

#### *Behavioural Observations*

The released plovers were searched for every day in the field using a car and mobile hide. When a focal plover was found, we recorded its behaviour for 30 min at 30 s intervals. Attempts were made to record the behaviour of focal plovers on at least two occasions before they found a new mate. Thirty minutes is sufficient to establish whether a plover is mated or not (Székely et al., 1999). We used behavioural categories of courting and self-maintenance behaviours that were previously developed for the Kentish plover (Székely et al., 1999). Courtship behaviours included: (1) courting: male plovers perform upright posture and high-stepping

movements and female plovers perform a lower head position; (2) copulations: courting pairs frequently copulated; (3) scraping: male and female plovers dig several scrapes in a territory; (4) fighting: focal individuals chase away intruders with puffed-out plumage and buzzing calls. Self-maintenance behaviours included: (1) feeding: individuals pick up food items followed by a short run; (2) preening: an individual groomed its own feathers. Mated individuals were identified based on courtship behaviours including courting, scrape ceremony and copulation (Urban et al., 1986; Hockey et al., 2005, see video of Kittlitz's plover courting behaviour in the Supplementary Material, Video S1). New pairs were checked for clutches every day, and eggs were measured as described above. Two observers recorded all behavioural observations (M.B. and J.E.P.).

### *Molecular Sexing*

As both plover species have sexually monomorphic plumage (Hockey et al., 2005; Urban et al., 1986), we used molecular sex typing to determine the sex of individuals (dos Remedios, Lee, Székely, Dawson, & Küpper, 2010). A small blood sample was taken from each adult's brachial wing vein, by puncturing, collecting drops of blood (about 25 µl) in capillary tubes, and storing this in Eppendorf tubes of Queen's Lysis Buffer. DNA was extracted from blood samples using the ammonium acetate extraction method (Miller, Dykes, & Polesky, 1988; Richardson, Jury, Blaakmeer, Komdeur, & Burke, 2001). For molecular sex typing, Z- and W-chromosome-specific genes were amplified via polymerase chain reaction (PCR) using the Z-002B/Z-002D primers (Dawson, 2007). For additional certainty in sex assignment, the W-chromosome-specific Calex-31 primers, developed in the genus *Charadrius*, were utilized (Küpper, Horsburgh, Dawson, Ffrench-Constant, Székely, & Burke, 2006). PCR amplification was conducted on a DNA Engine Tetrad 2 Peltier Thermal Cycler under the following conditions: 95 °C for 15 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 90 s, 72 °C for 60 s with a final extension of 60 °C for 30 min. Samples were visualized on an ABI 3730 automated sequencer. IR Dye-labelled tailed primers separated the products of Z-002B/Z-002D primers into either one (ZZ) or two (ZW) bands, indicating male or female, respectively. The W-specific Calex-31 product appeared as one band indicating female only. Images were scored using GeneMapper software version 4.1 (Applied Biosystems, Foster City, CA, U.S.A.). To maximize reliability, all samples were sexed using two markers.

Additionally, for 8% of samples (11 Kittlitz's plover and 10 white-fronted plover individuals) molecular sexing was repeated; in all cases, repetitions concurred with the original results.

#### *Data Processing and Statistical Analyses*

Date of mating was defined as the mean date between the date when a plover was last seen single and the first date it was seen with a new mate. Remating time was the difference between date of release (either on the day of manipulation or from captivity) and date of mating. The response variable, remating time, was analysed using generalized linear models (GLM) with Tweedie (1.5) error structure and a log link function (Smyth & Verbyla, 1999). The models investigated the effects of two main variables, species (Kentish, white-fronted and Kittlitz's plovers) and sex, and three additional fixed variables, type of manipulation (released in the field or released from captivity), release date and number of days in captivity (see Table 1). Dates were expressed as Julian dates, i.e. number of days since 1 January. Results of backward elimination based on Akaike's information criterion for small sample sizes (AICc) are presented for variable selection of the GLM models where lowest AICc score is the best supported model (Symonds & Moussalli, 2010; Table 1). Pairwise multiple comparisons were performed to compare mean differences in mating time between males and females of the three species using Fisher's least significant difference tests (LSD test).

Remating time was also analysed using survival analyses and these estimates are referred to as expected remating times (see rationale in Székely et al., 1999). In these analyses, the terminal event (outcome) was the occurrence of mating, defined as the first observation when a plover was seen with a mate. Several individuals did not find a new mate when we saw them for the last time, and these were treated as censored observations. First, we used a Gehan–Wilcoxon test to compare expected remating times curves (survival curves) for three species by sex. Survival curves were generated by the Kaplan–Meier method. Second, for each plover species a separate Cox regression model was constructed to investigate the probability of remaining single from the day of release (season), sex and their interaction as covariates (Table 2).

Courting behaviour was estimated as a percentage obtained from each 30 min sample. For individuals with several behavioural observations, we calculated the mean percentage of courting. Courting behaviour was analysed using GLMs with Tweedie (1.5) error structure and a logarithmic link function per individual plover. The model included two main factors, species of plover and sex, and three additional fixed variables, type of manipulation (released in the field or from captivity), release date and number of days in captivity. Model selection and statistical parameters estimated for each independent variable in the models are provided in the Appendix, Table A1. In addition, for each sex a separate GLM model was constructed to investigate the effect of three species of plovers on courting behaviour (Table 3). Pairwise multiple comparisons were performed to compare mean differences in courtship behaviour between males and females in the three species of plovers using LSD tests.

Finally, pair bond stability was analysed with a Fisher's exact test comparing the frequencies of mate replacement between white-fronted and Kittlitz's plovers. Data were analysed by using SPSS statistics for Windows version 19 (SPSS Inc., Chicago, IL, U.S.A.) and figures were made in R (R Development Core Team, 2008) using the package ggplot2 (Wickham, 2009).

#### *Ethical Note*

The experiments in Madagascar were approved by the Ministry of Environment, Forests and Tourism of the Republic of Madagascar (Research permit No: 053/11/MEF/SG/DGF/DCB.SAP/SCB of 11 March 2011 and 132/10/MEF/SG/DGF/DCB.SAP/SSE of 6 May 2010) and Madagascar National Parks (No: 398-10/MEF/SG/DGF/DVRN/SGFF of 18 May 2011). Blood sampling was also covered by these research permits. The blood transport permit was approved by Service de la Gestion de la Faune et de la Flore, Direction de la Valorisation des Ressources Naturelles, Ministère de l'Environnement et des Forêts Madagascar (authorization number 080N-EA06/MG11). The Kentish plover experiment was approved by the Turkish Ministry of Environment (see Székely et al., 1999). The Kittlitz's and white-fronted plovers are common breeding birds in much of Africa and Madagascar and they are not considered threatened by the IUCN (BirdLife International, 2012). Captive plovers were monitored daily and kept under

standard conditions (see Experimental Manipulation) to reduce their stress levels. In addition, translocated eggs coped with the natural breeding conditions of local clutches in the two plover populations (see above). The experiment was designed to reduce adverse effects on plover welfare and their local populations.

## Results

### *Between-Species Comparison*

Remating opportunities differed significantly between the three plover species (Fig. 1): white-fronted plovers mated significantly more quickly (median = 2.0 days, range 0.5–4.5 days,  $N = 12$ ) than both Kittlitz's plovers (median = 5.1 days, range 1.0–11.5 days,  $N = 16$ ) and Kentish plovers (median = 6.3 days, range 0.5–47.5 days,  $N = 34$ ; Table 1).

These results remained consistent when we used survival analyses that also included the individuals that were not successful in finding a new mate (Fig. 2, Table 2; see Methods). The proportion of plovers remaining single was significantly lower for the white-fronted plover (median = 4 days,  $N = 14$ ) than both the Kittlitz's plover (median = 14.6 days,  $N = 33$ ) and Kentish plover (median = 13.4 days,  $N = 59$ ; testing the three species, Wilcoxon–Gehan test:  $\chi^2_2 = 16.316$ ,  $P < 0.001$ ).

### *Between-Sexes Comparison*

A significant species\*sex interaction suggested a sex-biased difference in remating opportunities (Table 1). Female Kittlitz's plovers took significantly longer to mate (median = 6.5 days, range 3.5–11.5 days,  $N = 6$ ) than males (median = 3.3, range 1.0–7.5 days,  $N = 10$ ; LSD test: pairwise mean difference = -0.66,  $df = 1$ ,  $P = 0.047$ ) whereas the opposite was found in the Kentish plover (Székely et al. 1999). However, male and female remating times were not significantly different in white-fronted plovers (male: median: 2.0 days, range 0.5–3.5 days,  $N = 6$ ; female: median: 2.0 days, range 1.0–4.5 days,  $N = 6$ , LSD test: pairwise mean difference = -0.11,  $df = 1$ ,  $P = 0.823$ ).

These results remained consistent when we used survival analyses (Table 2): the proportion of female Kittlitz's plovers remaining single was higher than that of males (male median: 11.0 days,  $N = 17$ ; female median: 21.0 days,  $N = 16$ ; Fig. 2),



whereas the proportions of single males and females were not significantly different in the white-fronted plover (male median: 3.0 days,  $N = 7$ ; female median: 4.0 days,  $N = 7$ ; Fig. 2). Remating time increased over the season only for female Kentish plovers (Cox regression:  $\chi^2_1 = 7.66$ ,  $P = 0.014$ ), suggesting an influence of time of breeding season on mating opportunities in the Kentish plover, although this was not the case in the other two species (Table 2).

### *Courtship Behaviour*

Courtship behaviour had a significant species\*sex interaction (Appendix Table A1, Fig. 3). Courtship behaviour by males differed significantly between species (Table 3) with male Kentish plovers spending significantly more time on courtship than males of the other plover species, whereas courtship behaviour by females did not differ between species (Table 3). In contrast to the Kentish plover, which exhibited male-biased courtship behaviour (LSD test: pairwise mean difference = -3.29,  $df = 1$ ,  $P = 0.005$ ), males and females of the other two species spent comparable times on courtship (LSD test white-fronted plover: pairwise mean difference = 1.06,  $df = 1$ ,  $P = 0.252$ ; LSD test Kittlitz's plover: pairwise mean difference = -0.36,  $df = 1$ ,  $P = 0.679$ ; Fig. 3).

### *Pair Bonds*

The new pair bonds in experimentally manipulated white-fronted plovers were significantly weaker than in Kittlitz's plover: in 12 white-fronted plovers that remated after their former partner was removed (six males, six females), all experimentally induced pair bonds were replaced by the original mates after they were released from captivity. In contrast, in 16 Kittlitz's plovers that remated after their former partner was removed (10 male and six female), none were replaced by their former mates when these were released from captivity (Fisher's exact test: mate replacement in white-fronted plover = 12,  $N = 12$ ; mate replacement in Kittlitz's plover = 0,  $N = 16$ ;  $P < 0.001$ ).

## **Discussion**

These experiments provided four key results. First, they show that mating opportunities differed significantly between these closely related species. This result is striking because two of these plover species (white-fronted and Kittlitz's) breed in

the same habitat in Madagascar, and therefore ecological factors are unlikely to explain the differences in remating opportunities. The rapid remating of white-fronted plovers, a pattern we did not expect, suggests that there is a large pool of floating individuals that can rapidly move in to pair up with unmated individuals. Consistent with the latter argument, both male and female white-fronted plovers stayed in the same territory, and new individuals moved in to replace the removed mates. Mating opportunities seem to be an important factor in the evolution of breeding systems across a range of species (Balshine-Earn & Earn, 1998; Magrath & Komdeur, 2003). For example, as in the white-fronted plover, male dunlin, *Calidris alpina*, were rapidly replaced by other males after experimental removal from their breeding territories (Holmes, 1970; Pitelka, Holmes, & Maclean, 1974). In the European starling, *Sturnus vulgaris*, after mating opportunities were increased by the provision of additional nestboxes, males increased their mating effort to attract additional mates and also reduced parental care effort (Smith, 1995), and in St Peter's fish, *Sarotherodon galilaeus*, males and females were more likely to desert the offspring when remating opportunities were increased experimentally (Balshine-Earn & Earn, 1998).

Second, we found a sex bias in remating opportunities: the male-biased remating opportunities in Kittlitz's plover were the opposite of those found in the Kentish plover (Székely et al., 1999), whereas in white-fronted plovers remating opportunities did not differ between males and females. As far as we are aware, our study is the first to demonstrate experimentally differences in sex-biased remating opportunities between closely related species in wild populations. Sex-biased mating opportunities may emerge in two ways. One explanation is that the ratio of sexually active males to females (OSR) may not be at parity. OSR may be biased because of differences in the reproductive schedules of males and females ('time in': time spent in the mating pool, sexually active; 'time out': time spent out of the mating pool, not sexually active) or biased adult sex ratios (ASR; Donald, 2007). Evidence suggests that OSR can vary because of mating and parental care activities (Forsgren et al., 2004; Symons, Svensson, & Wong, 2011; LaBarbera, Lovette, & Llambías, 2011; Canal, Jovani, & Potti, 2012). In addition, recent studies found substantial differences in ASR between closely related shorebird species (Liker et al., 2013). Further studies are needed to separate whether biased remating opportunities emerge

via different reproductive scheduling or biased ASR in plovers. For one of these species, the Kentish plover, a demographic study confirmed male-biased ASR (Kosztolányi et al., 2011), although ASR has not been estimated for the white-fronted and Kittlitz's plovers. Alternatively, sex-biased mating opportunities may arise from differences in the willingness of males and females to remate. For instance, the postbreeding refractory periods, the recovery phase spent preparing for another breeding attempt, may differ between males and females (Balshine-Earn & Earn, 1998; Cantoni & Brown, 1997): females typically need more time to recover than males. However, the latter explanation is unlikely, since the adult plovers used in our experiments had breeding efforts interrupted and sought new mates shortly after removal of their mate (or on release from captivity). Furthermore, several female Kentish plovers remated within less than a day, a pattern that is inconsistent with the explanation that females need more time to recover than males.

Third, male courtship behaviour differed between the three species, since male Kentish plovers spent more time on courtship than male white-fronted and Kittlitz's plovers. This pattern is consistent with the explanation that ASR is male-biased in the Kentish plover. The significance of this result is that courtship behaviour is variable between closely related species and suggests that ASR, and in turn the OSR, is probably related to the intensity of mating competition in males. Consequently, a comparable intensity of courtship behaviour was observed between the sexes in the Kittlitz's and white-fronted plovers. The latter result indicates that males and females may compete similarly for available mates, suggesting that they do not have conventional sex roles: male-male competition and female choice for mates (Vincent, Ahnesjö, & Berglund, 1994). Variation in resources for breeding has also been suggested to influence OSR, and, in turn, the intensity of mating competition (Forsgren, Kvarnemo, & Lindstrom, 1996). Availability of breeding territories, for example, may affect the OSR, since the sex that holds the territories will be limited by scarcity of nest sites. In a sand goby, *Pomatoschistus minutus*, population for instance, nest site abundance can influence the intensity of male mating competition (Forsgren et al., 1996). Hence, the dynamic of OSR and, in turn, mating competition is probably modulated by both ASR and resource availability (Kvarnemo & Ahnesjö, 1996, Forsgren et al., 2004).

Finally, the new pair bonds were significantly weaker in white-fronted plovers than in Kittlitz's plover suggesting that the former species exhibits long-term pair bonds whereas the latter has short-term pair bonds. Mate fidelity may emerge in two ways. On the one hand, former mates may actively seek out each other and prefer to mate with each other. On the other hand, mate fidelity may emerge via site fidelity: white-fronted plovers are highly territorial (Lloyd, 2008), and therefore upon release from captivity, individuals return to their former territories and chase out their former partner's new mate. Established pairs may prefer to reunite because of the fitness benefits in terms of synchronization of behavioural and physiological characteristics such as defence of breeding territories, courtship behaviours, laying date, incubation and chick raising, which have been shown to improve with time and experience of the pair (Bried, Pontier, & Jouventin, 2003; Rowley, 1983). An experiment carried out in the bearded reedling, *Panurus biarmicus*, found that long-term pair bond formation improved coordination of breeding activities and reproductive success (Griggio & Hoi, 2011). In another example, newly formed pairs (because of either divorce or loss of a mate) had lower reproductive success than established pairs in black turnstones, *Arenaria melanocephala* (Handel & Gill, 2000). Improved breeding with increased experience is also well known in geese, albatrosses and other long-term pair-bonding animals (Angelier, Weimerskirch, Dano, & Chastel, 2006; Black, 2001). Overall, the consequences of pair bond and site fidelity for mating opportunities could be significant since the ability of an individual to mate may be limited by its access to mates and breeding sites.

In conclusion, using an experimental approach we found significant differences in remating opportunities between closely related plover species. As mating opportunity is linked to OSR and ASR, our work suggests that substantial variation in OSR (and possibly ASR) is exhibited among closely related species. Such variation may influence the direction and intensity of competition in males and females for mates and breeding territories. These differences in OSR, in turn, may facilitate different intensities of sexual selection and induce different mating systems and patterns of parental care.

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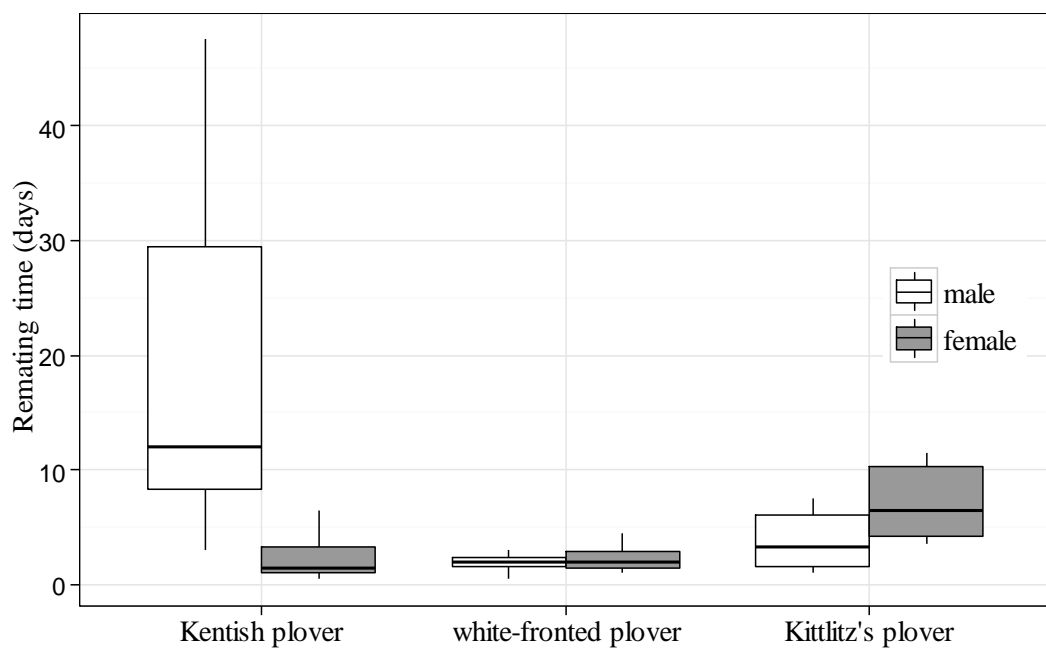
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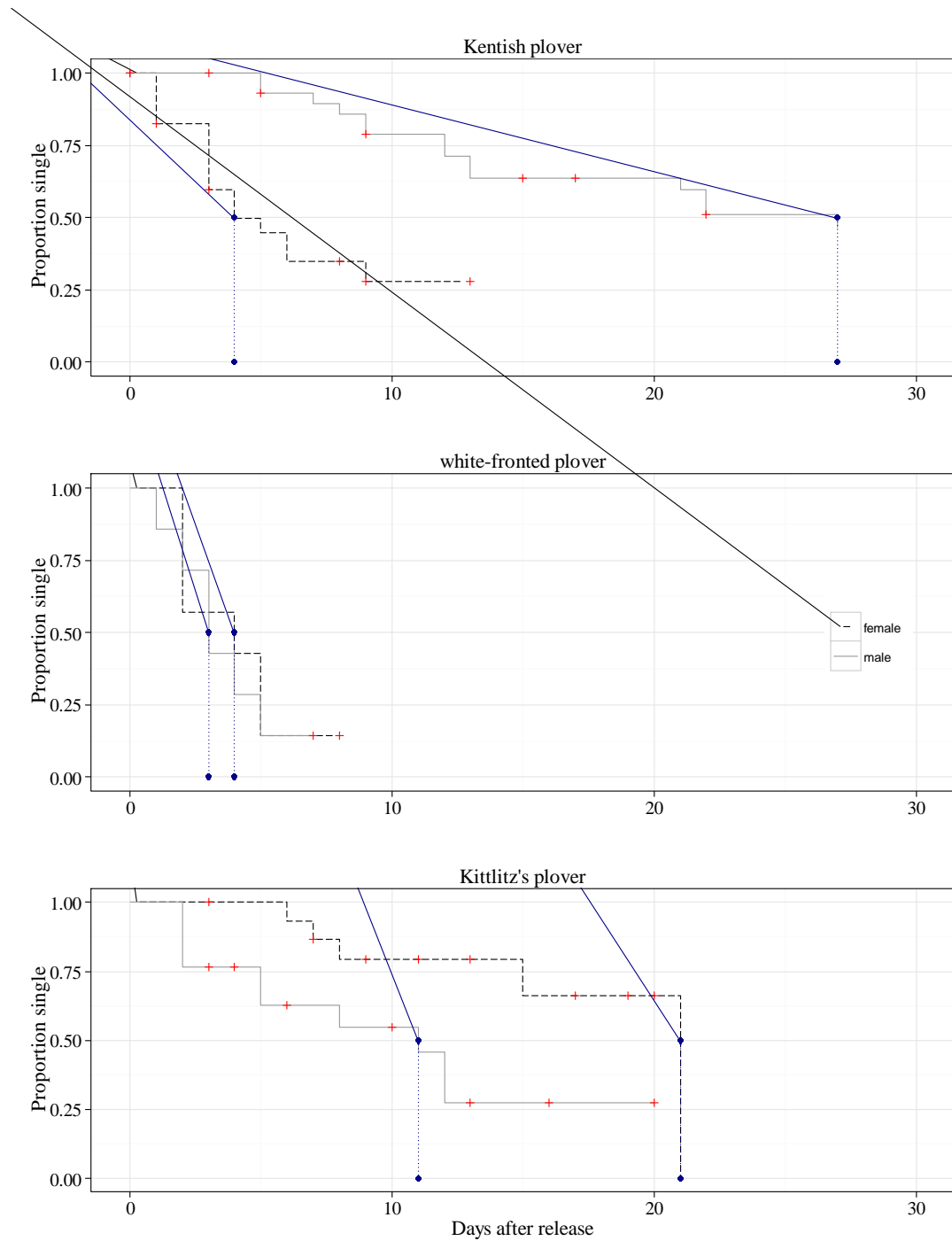
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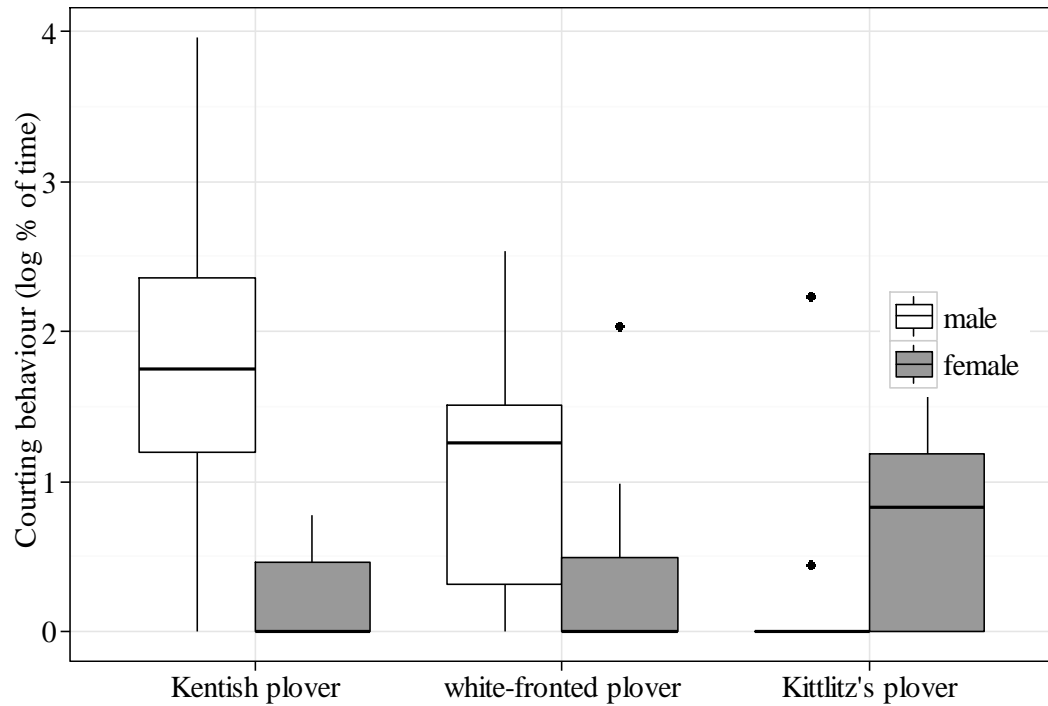
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**Figure 1.** Remating times in three plover species. The lower and upper borders of the box are lower and upper quartiles, respectively, the horizontal bar is the median and whiskers represent the lowest and highest observations.



**Figure 2.** Proportion of males and females remaining single in three plover species: Kentish plover, white-fronted plover and Kittlitz's plover. Dotted lines and blue points show the expected mating time of males and females after release. Number of individuals: 32 male and 27 female Kentish plovers; seven male and seven female white-fronted plovers; and 17 male and 16 female Kittlitz's plovers.



**Figure 3.** Courtship behaviour in three plover species. The lower and upper borders of the box are lower and upper quartiles, respectively, the horizontal bar is the median and whiskers represent the lowest and highest observations. Circles denote outliers that are between the first and third interquartile from the nearer edge of the box.

**Table 1.** Remating times (response variable) of males and females in three species of plover

Independent variable	Full model (AICc = 346.004)			Best model (AICc = 341.088)		
	Wald $\chi^2$	df	P	Wald $\chi^2$	df	P
(Intercept)	38.596	1	<0.001	49.365	1	<0.001
<b>Between-species comparison</b>						
Species	11.248	2	0.004	11.595	2	0.003
<b>Between-sexes comparison</b>						
Sex	4.072	1	0.044	3.974	1	0.046
Species * sex	39.65	2	<0.001	47.620	2	<0.001
Manipulation	0.290	1	0.59	-	-	-
Release date	4.818	1	0.028	5.007	1	0.025
Captive days	0.646	1	0.422	-	-	-

GLMs were used to analyse mating time using Tweedie (1.5) error structure and a log link function. Model selection was carried out using the Akaike information criterion for small sample sizes (AICc). Manipulation refers to whether a plover was kept in captivity or not. Release date refers to the date when a plover was released to find a new mate (Julian dates). Captive days are the number of days that a plover was kept in captivity.

## MATING OPPORTUNITIES

**Table 2.** Remating time in plovers based on Cox proportional hazard models, to examine the relationship of the survival distribution which includes censored observations

Species	Variable	B	Wald $\chi^2$	df	P
Kentish plover	Sex	1.541	12.07	1	0.001
	Release date	-0.024	6.073	1	0.014
White-fronted plover	Sex	-0.18	0.083	1	0.77
	Release date	0.002	0.004	1	0.95
Kittlitz's plover	Sex	-1.342	4.864	1	0.027
	Release date	-0.01	0.088	1	0.767

For each species a separate model was constructed. Number of individual Kentish plovers, mated = 34, censored = 19; white-fronted plover, 12, 2; Kittlitz's plover, 16, 17, respectively.

**Table 3.** Courtship behaviour (response variable: % of time courting) in three plover species

Sex	Variables	Wald $\chi^2$	<i>df</i>	<i>P</i>
Male	(Intercept)	13.176	1	< 0.001
	Species	10.689	2	0.005
Female	(Intercept)	0.155	1	0.694
	Species	1.437	2	0.487

GLMs were used to analyse percentage of time courting using Tweedie (1.5) error structure and a log link function. Model selection was carried out using the Akaike information criterion for small sample sizes (AICc).



## Appendix

**Table A1.** Courtship behaviour in plovers (response variable: proportion of time courting)

Independent variable	Full model (AICc = 269.035)			Best model (AICc = 261.316)		
	Wald $\chi^2$	df	P	Wald $\chi^2$	df	P
(Intercept)	2.215	1	0.137	2.914	1	0.088
Species	0.621	2	0.733	0.791	2	0.673
Sex	5.713	1	0.017	5.381	1	0.020
Species * sex	6.084	2	0.048	6.329	2	0.042
Manipulation	0.057	1	0.811	-	-	-
Release date	0.056	1	0.813	-	-	-
Captive days	0.477	1	0.490	-	-	-

GLMs were used to analyse courtship behaviour using Tweedie (1.5) error structure and a log link function. Model selection was carried out using the Akaike information criterion for small sample sizes (AICc).

## CHAPTER FOUR

# **Mating Opportunities Predict Different Spatial and Social Interactions in Mate Searching of Two Malagasy Shorebirds**

Jorge E. Parra, Kate Ashbrook, Marcela Beltrán, Sama Zefania and Tamás Székely

*Manuscript*

*Author's contributions*

**JEP:** study design, data collection, statistical analysis, manuscript preparation

**KA:** statistical analysis

**MB:** data collection in the field

**SZ:** data collection in the field

**TSz:** study design, manuscript improvement

**Abstract**

A fundamental, yet rarely, investigated component of breeding system variation is mating opportunity, the frequency with which an unmated animal finds a mate. Mating opportunity may relate to the ratio of sexually active males to females, i.e. the operational sex ratio (OSR). Experimental assessments of mating opportunity is a powerful approach estimating mate availability since the animals themselves seek out new mates and thus directly revealing mate availability. It has recently been established that mating opportunities are different between two closely-related shorebird species: female Kittlitz's plovers (*Charadrius pecuarius*) had longer remating times than male Kittlitz's plovers, whereas in white-fronted plover (*C. marginatus*) remating times were comparable and short between males and females. Here we test whether OSR predict mating opportunities in a natural avian system. The observed difference in mating opportunity predicts (i) sex-biased mate searching behaviour in the Kittlitz's plover, whereas similar mate searching behaviour in the white-fronted plover and (ii) more intensive search effort in the white-fronted plover than the Kittlitz's plover. We tested these predictions in sexually-active males and females of both plover species by studying their spatial and social interactions when searching for a mate. We found that OSR is predictor of mating opportunities. Male and female white-fronted plovers had similar space use during mate searching; in contrast, Kittlitz's plover females searched larger areas for mates than male Kittlitz's plovers. In addition, mate-searching Kittlitz's plovers have access to a great number of conspecifics than mate-searching white-fronted plovers. Therefore, mate searching behaviour differs between closely-related plover species consistently with difference in mating opportunities. These differences may be a consequence of the number of mates and breeding sites available within a population, influencing the OSR, and in turn, mating and parental care patterns.

**Keywords:** mate searching, operational sex ratio (OSR), adult sex ratio (ASR), parental care, breeding systems, shorebirds, Malagasy plovers.

## Introduction

Mate searching behaviour is the effort expected by males and/or females to increase mate-encounter rates (Kokko & Wong 2007). It occurs when individuals (males or females) are looking for mating partners in a population. Searching for a mate, however, is costly in terms of time and energy invested in physical movements, production of pheromones, acoustic signals or other sensory mechanisms that increase mate encounters (Barnes 1982; Duvall & Schuett 1997; Byers et al. 2006; Kasumovic et al. 2006; Guevara-Fiore et al. 2010). Thus, mate searching behaviours may reduce investment in other components of fitness such as survival and fecundity. For example, mate searching can increase the risk of death due to predation or strong competition for mates, and reduce female egg production and future male mate competition abilities (Berglund 1993; Acharya 1995; Liker & Székely 2005; Tuni & Berger-Tal 2012). Moreover, there is likely to be sexual conflict over searching effort. The sex with less investment in mate searching will take advantage of the mate searching effort incurred by the other sex (Parker & Partridge 1998; Kokko & Wong 2007).

Males are usually the searching sex as they invest less in young (Clutton-Brock & Vincent 1991). Thus, it has been suggested that males stay longer in the mating pool than females. Males can be in one of two states: searching for a mate or breeding, whereas females can be in one of three states: receptive (searching), non-receptive and breeding. Theoretical models, however, suggest similar capacities in mate searching behaviour between males and females despite sexual difference in the potential reproductive rate (Kokko & Wong 2007). Therefore, mating opportunities have been suggested to play a key role for sex differences in mate searching behaviour (McNamara et al. 2000; Kokko & Jennions 2008). For instance, female mate searching behaviour is also common in fishes, birds, anurans and some insects (Slagsvold et al. 1988; Forsgren 1997; Uy et al. 2000; McCartney et al. 2012; Myhre et al. 2012). If more males than females are available in the mating pool, males should search more intensely for a mate than females. The opposite will be true for females, as sperm is limited a great mate searching effort by females is expected. Patterns of mate searching behaviour, then, should vary according to the ratio of sexually-active males to fertilizable females (Emlen & Oring 1977), the operational sex ratio (OSR). Variation in the OSR, and in

turn mate searching behaviour, might emerge from differences in reproductive activities of males and females. That is, the time that each sex spends in parental care and the recovery phase after a breeding attempt have been suggested to influence in the ratio of sexually-active males to females in the mating pool (Balshine-Earn & Earn 1998; Owens 2002).

Few studies, however, have investigated how parental care patterns and mating opportunities influence sex roles in mate searching. On the one hand, it is predicted that the sex with lower parental care investment will be the searching sex for a mate. In contrast, mate searching effort should be similar for males and females if they are equally involved in rearing the offspring. On the other hand, biased mating opportunities can also influence the sex roles on mate searching independently of parental investment by the sexes. Populations with male-biased mating opportunities can increase male searching effort and the opposite is expected as well. If sexes have similar mating opportunities, it is expected similar mate searching effort for both sexes.

Here, we tested these predictions using sexually-active males and females of two shorebird species with different parental care systems and mating opportunities. Specifically, we used spatial and social interaction analyses to study the mate searching behaviours in two small plovers, the white-fronted plover, *Charadrius marginatus*, and the Kittlitz's plover, *Charadrius pecuarius*. White-fronted plovers exhibit biparental care, whereas Kittlitz's plovers are reported to have uniparental care by the male (see chapter three). The white-fronted plover is a monomorphic species distribute along sandy shores of lakes, rivers and coasts. Breeding pairs maintain and defend territories for foraging and nesting against neighbours and other shorebird species along shores. Pairs can make several breeding attempts within a breeding season if clutches are lost. Both parents share incubation and brood care (see chapter two). They have high mate and site fidelity within and between years in South Africa populations (Lloyd 2008). Kittlitz's plover is a monomorphic species found in short grass and open habitats often near to bodies of water. Pairs breed in vague colonies where they defend small territories until young hatch. Both parents share incubation but brood attendance has been reported by the male (see chapter two).

A previous mating opportunity study of the white-fronted plover and the Kittlitz's plover in Madagascar found three main results (Parra et al. 2014). First, remating time of the white-fronted plover was significantly shorter than remating time of the Kittlitz's plover. Second, female Kittlitz's plover remating time was longer than male Kittlitz's plover remating time whereas no difference in remating time between sexes was found in the white-fronted plover. Third, pair bond in the white-fronted plover was stronger than in the Kittlitz's plover since previous pairs in the white-fronted plover have always remated whereas the previous pair bonds in the Kittlitz's plover was never reinstated.

Based on Parra *et al.* (2014), we derived the following predictions. First, we predicted sex-biased mate searching behaviour in the Kittlitz's plover and no bias in the white-fronted plover, since females had higher mating opportunities than males Kittlitz's plovers, whereas both sexes had comparable mating opportunities in the white-fronted plover. Second, we predicted more intense mate searching behaviour in the Kittlitz's plover than in the white-fronted plover given that mating opportunities are male-biased in the Kittlitz's plover and not biased in the white-fronted plover. We tested these predictions investigating movements and, using the overlap between individual home ranges, we derived social networks during mate searching of males and females of the white-fronted and the Kittlitz's plovers used in the mate removal experiment of Parra and colleagues (2014).

## Methods

### *Study species and study sites*

Mate searching behaviour was studied in the Kittlitz's plover and the White-fronted plover in the South-west coasts of Madagascar. The study was carried out in conjunction with experimental assessment of mating opportunities of both plovers (Parra et al. 2014). Kittlitz's plover was studied between February and May 2010 in Andavadoaka region (22°02' S, 43°39' E). There were an estimated 300 breeding Kittlitz's plovers around grasslands and saltmarshes. White-fronted plover was studied between April and June 2011 in Lake Tsimanampetsotsa National Park (24° 3' S, 43°44' E). Approximately 150 white-fronted plovers breed along sandy shores, short grass and saltpans of the salty lake. In total, 18 pairs (36 individuals) of the Kittlitz's plover and 14 pairs (28 individuals) of the White-fronted plover were

captured for both the mating opportunities experiment and mate searching study. The two species of plover were caught on the nest with funnel traps. Their morphological traits (body mass, tarsus length, wing length and bill length) were measured with a sliding caliper, wing ruler, and spring balance (see details of materials and methods in Kentish plover field guide, Székely et al. 2007a). Each plover was ringed with an individual combination of colour rings and a numbered SAFRING metal ring from the University of Cape Town in South Africa. In the field, determining the sexes of both species of plovers was difficult (Urban et al. 1986; Hockey et al. 2005), thus a small blood sample was taken from the wing brachial vein of each adult, by puncturing and collecting drops of blood (~25 ul) with capillaries and storing blood in tubes with Queen's Lysis Buffer for molecular sexing. Molecular sexing was carried out in NERC-Biomolecular Analysis Facility at the University of Sheffield. (for details see Dos-Remedios et al. 2010; Parra et al. 2014).

#### *Unmated sexually-active individuals*

To quantify plover movements and behaviours from single to mated, unmated individuals were created using the experimental manipulation described in Székely et al. (1999). Briefly, parents of both species were capture on their nest; one parent was chosen at random (male or female) and released immediately after ringing, measuring and blood sampling. The other parent was taken into captivity (see below). Only pairs incubating two eggs (modal clutch size for the two species) were manipulated. Egg length and breadth were measured with a sliding calliper, and the number of days eggs had been incubated for was estimated based on the floatation stage of the egg in a transparent jar with clean water (see details of methodology in Kentish plover field guide, Székely et al. 2007). Eggs were distributed into other plovers' clutches with only one egg and at approximately the same stage of incubation.

Removed plovers were transported to a purpose-built aviary near our field camp at both study sites. The aviaries had four units for the Kittlitz's plover and six units for the White-fronted plover. Each unit was built of a 1 m x 1.5 m x 1.5 m (height x length x width) wood frame with chicken mesh (13 x 13 mm.). To provide shade for the captive birds, we covered the aviary outside by papyrus, *Cyperus sp.*, and 50 cm of cloth at the base of the mesh inside the units. The floor of the aviaries

was sand from the shore of beaches or lakes, as found at the plovers' habitats. Captive plovers were fed with appropriate food and water three times per day every day to maintain their good health using a high protein meals: dried insects for wild birds (dried mealworms, dried earthworms, gammarus shrimp and dried waterfly - Natures Grub ©), bird supplement vitamins (Vitacombex V) and pinhead oatmeal (Prosecto Insectivorous™ - Haith's ©). Since plovers are insectivorous, captive plovers were also supplied with fresh beetles twice a day using pit fall traps set-up in the salt-marsh. Captive plovers were released after their former mate found a new mate (see Parra et al. 2014), and were measured, ringed, with the time and date of release recorded.

#### *Relocations and behaviour from single to mated*

The released plovers were searched for every day in the salt marshes and around lakes using a car and a mobile hide (Székely et al. 2003), so as to not disturb their behaviours. When a focal plover was found geographical coordinates of each plover were taken with a handheld GPS receiver (Garmin e-Trex H) and instantaneous samples of their behaviour were recorded (see Parra et al. 2014). Attempts were made to record the location and behaviour of focal plovers several times both before and after they found a new mate (Appendix, Table 1A), although we were unable to relocate some plovers before and/or after mating. Mated individuals were identified by courting behaviours such as scrape ceremony, nest-scrape and copulation (see video in Parra et al. 2014). New pairs were checked for clutches every day and eggs were measured as described above.

#### *Spatial and social interaction analyses*

Hawth's Analysis Tool, an extension for ArcGIS 9.1 (ESRI 2008), was used for spatial analyses. The tool produced a triangular matrix of distances between original (first nest location) and secondary territories of the two species by each sex. When the second nest was not found, after manipulation, a centroid point was generated by a polygon of the relocations, which was used as the secondary territory's point in the distance matrix.

Package "adehabitatHR" for R (Calenge 2006) was used to analyse the use of space for single plovers searching for a mate by using kernel method. First,



utilization distribution (UD) of the space of 11 white-fronted plovers and 16 Kittlitz's plovers was calculated as the probability density of relocations (Appendix, Figure 1A). The smoothing parameter was computed by least square cross validation (LSCV) method. Second, 0.95 isopleth was estimated from the output kernel-UD and the mate searching area was calculated from the boundary containing 95% of the area of the surface, the 0.95 isopleth. Third, plovers long distance path were calculated with the package “adehabitatLT” for R. It estimates the accumulative distance between relocations of plovers searching for a mate. Finally, kernel overlap between single plovers and other sample of plovers in the population was calculated by kernel home-range method. We use the utilization distribution overlap index (UDOI) which estimates the amount of overlap between two plovers using the same space. A value less than one indicates less overlap whereas a value more than one indicates higher overlap.

We used generalized linear models (GLM) with Gaussian error structure and identity link function, to test whether species, sex and their interaction influence the mate searching behaviour of plovers (distance from previous territory, mate searching area and distance path of plovers); with three additional variables: days tracked, number of relocations and type of manipulation (released in the field or released from captivity). There was a significant difference between species in their mate searching behaviour (see Results), so we analysed the two species separately. Box-Cox transformation was used for the response variables to have approximately normal and heteroscedastic errors. Data were analysed using the package “car” (Fox and Weisberg, 2011) and MASS (Venables and Ripley, 2002) for R (R Development Core Team, 2010). Figures were made in R (R Development Core Team, 2010) using the package ggplot2 (Wickham, 2009).

## Results

### *Breeding territories and mate searching behaviour*

Neither species nor sexes differed significantly in the distance moved from starting territories to new territories (GLM, species x sex:  $t = 0.188$ ,  $P = 0.85$ ; species:  $t = -0.042$ ,  $P = 0.96$ ; sex:  $t = -0.37$ ,  $P = 0.71$ ; Figure 1).

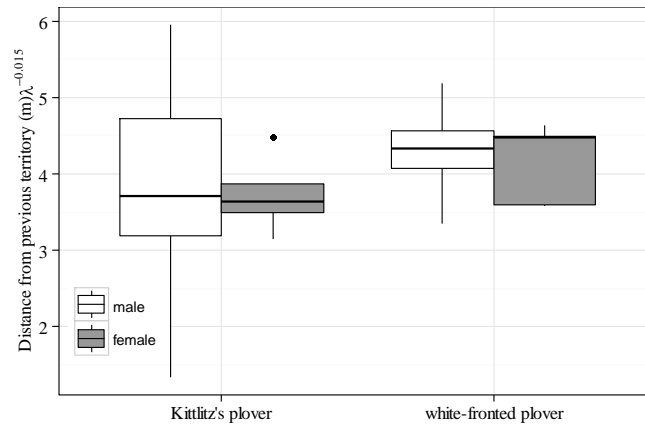
The mate searching areas differed significantly between the two plover species (Figure 2): Kittlitz's plovers used larger areas (mean = 147952.91 m<sup>2</sup>, sd ± 158715.94 m<sup>2</sup>, N = 16) than white-fronted plovers (mean = 33023.59 m<sup>2</sup>, sd ± 45372.18 m<sup>2</sup>, N = 16; Table 1). Furthermore, we found that mate searching behaviour between the sexes differed between the two plover species. In Kittlitz's plover, females used larger areas (mean = 187787.84 m<sup>2</sup>, sd ± 178270.13 m<sup>2</sup>, N = 11) than males (mean = 60316.08 m<sup>2</sup>, sd ± 32414.22 m<sup>2</sup>, N = 5; GLM:  $t = 2.47$ ,  $P = 0.027$ ; Figure 2). In contrast, mate searching area size was similar between male and female white-fronted plovers (GLM:  $t = 1.27$ ,  $P = 0.23$ ; Figure 2).

The distance path followed by plovers searching for mates differed significantly between the two species (Figure 3): the distance travelled by Kittlitz's plovers was larger (mean = 3701.31 m, sd ± 3162.08 m, N = 16) than by white-fronted plovers (mean = 979.55 m, sd ± 638.4 m, N = 16; Table 2). However, there was no significant difference between sexes and no interaction between species and sex (Table 2).

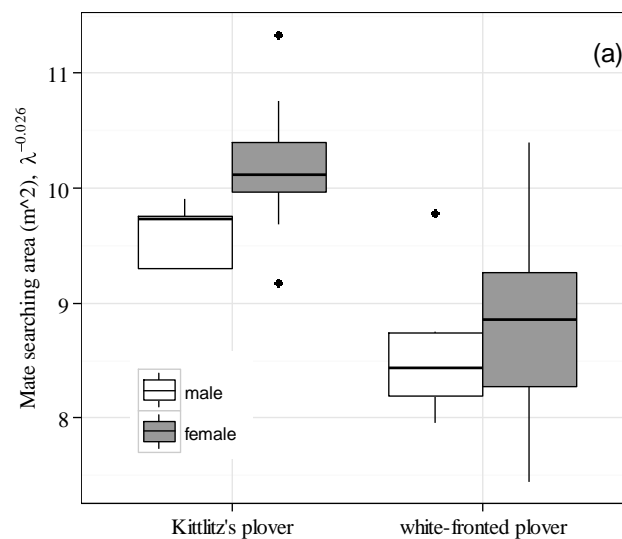
#### *Social encounter*

Overlap between the home ranges of individuals differed between species and sexes (Figure 4). The home ranges of individual Kittlitz's plovers overlapped more with conspecifics than did white-fronted plovers (GLM, species:  $t = -4.45$ ,  $P = 0.0001$ ). Also, in Kittlitz's plover, the home ranges of individual females overlapped with conspecifics marginally more than male home ranges (GLM:  $t = 1.96$ ,  $P = 0.059$ ; Figure 4). Finally, we visualised likelihood of encounters with neighbouring individuals by mapping their home ranges. In Kittlitz's plover, males and females had a higher probability of interacting with a large number of conspecifics (Figure 5). In contrast, white-fronted plovers were more likely to interact with a smaller number of local individuals, and this was true for both males and females (Figure 6).

## MATE SEARCHING



**Figure 1.** Distance from previous territory in two plover species. The data are Box-Cox transformed and normalized using the parameter  $\lambda$ . The lower and upper borders of the boxes are lower and upper quartiles, respectively, the horizontal bar is the median and whiskers represent the lowest and highest observations. Circles denote outliers that are between the first and third interquartile from the nearer edge of the box.

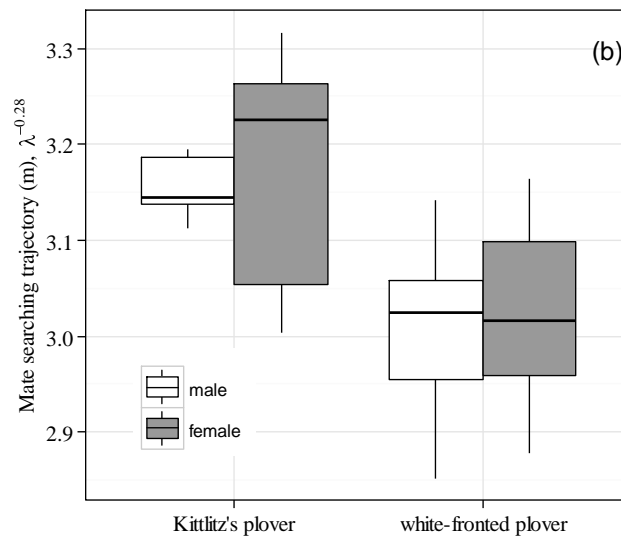


**Figure 2.** Mate searching area in two plover species. The data are Box-Cox transformed and normalized using the parameter  $\lambda$ . The lower and upper borders of the boxes are lower and upper quartiles, respectively, the horizontal bar is the median and whiskers represent the lowest and highest observations. Circles denote outliers that are between the first and third interquartile from the nearer edge of the box.

**Table 1.** Mate searching area (response variable) of male and female Malagasy plovers. Estimated effect sizes  $\pm$  SE (standard errors)

Independent variable	Coefficient	$\pm$ SE	t-test	<i>P</i>
Intercept	9.59	0.24	39.98	<0.001
Species	-1.16	0.24	-4.77	<0.001
Sex	0.52	0.25	2.1	0.044

GLM were used to analyse mate searching area using Gaussian error structure and identity link function. The data are Box-Cox transformed and normalized.

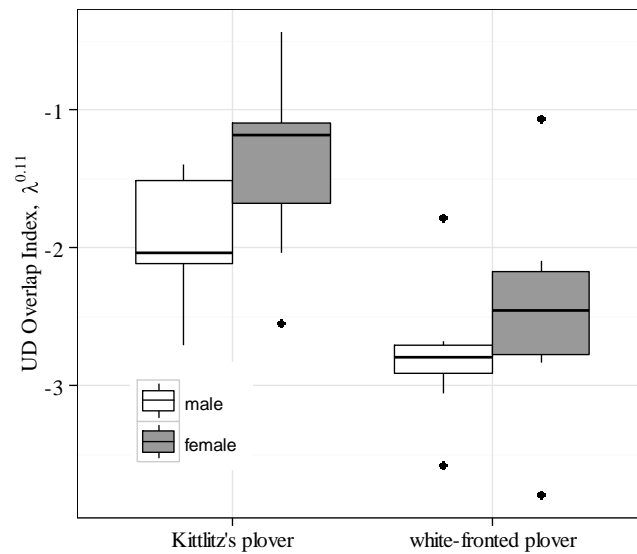


**Figure 3.** Mate searching trajectory in two plover species. The data are Box-Cox transformed and normalized using the parameter  $\lambda$ . The lower and upper borders of the boxes are lower and upper quartiles, respectively, the horizontal bar is the median and whiskers represent the lowest and highest observations. Circles denote outliers that are between the first and third interquartile from the nearer edge of the box.

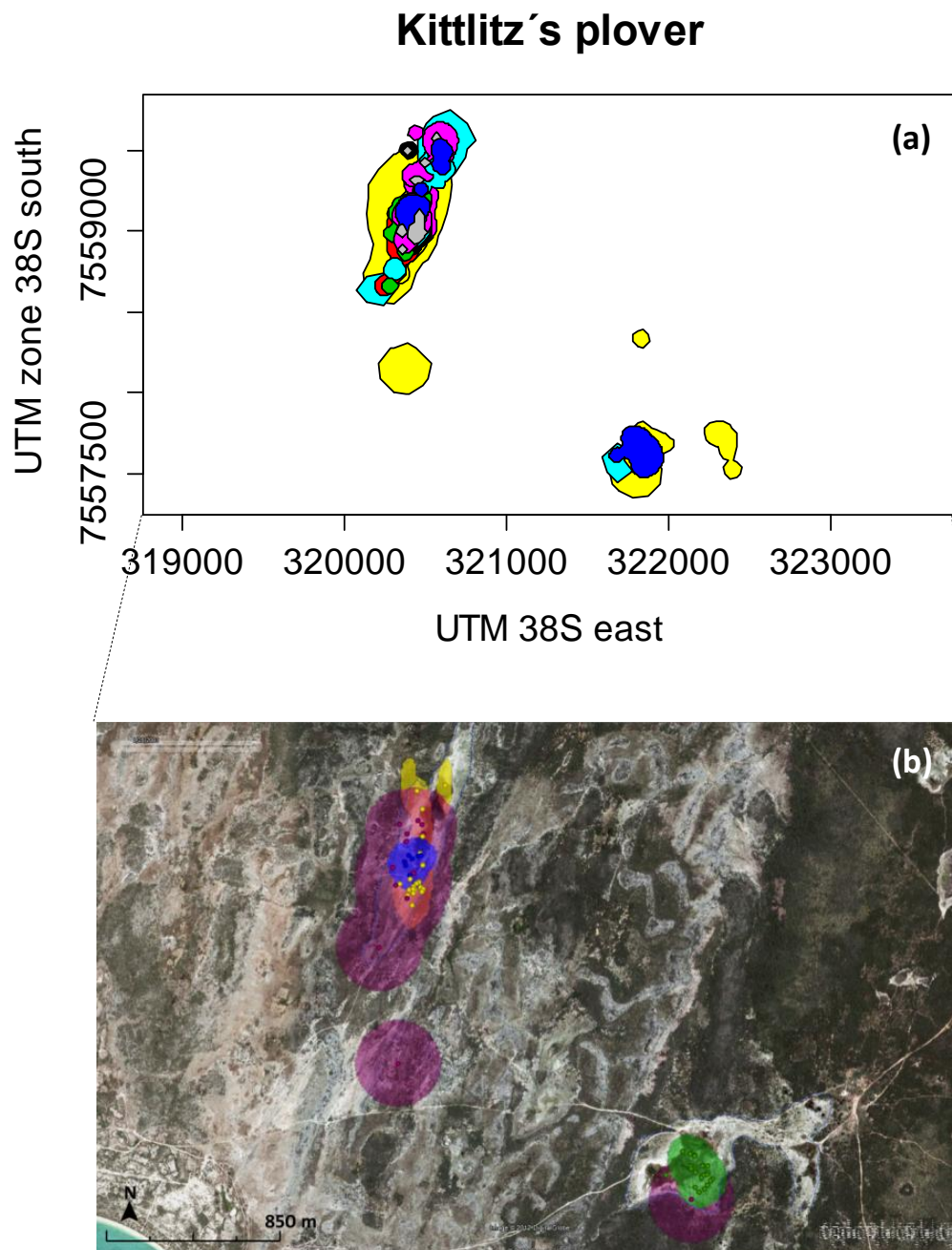
**Table 2.** Mate searching trajectory (response variable) of male and female Malagasy plovers. Estimated effect sizes  $\pm$  SE (standard errors).

Independent variable	Coefficient	$\pm$ SE	t-test	<i>P</i>
Intercept	3.07	0.087	35.4	<0.001
Species	-0.16	0.057	-2.87	0.008
Sex	0.024	0.044	0.56	0.58
Species * sex	0.009	0.059	0.152	0.88
Manipulation	-0.039	0.032	-1.23	0.23
Days tracked	-0.002	0.001	-1.51	0.144
No relocations	0.009	0.002	3.74	0.001

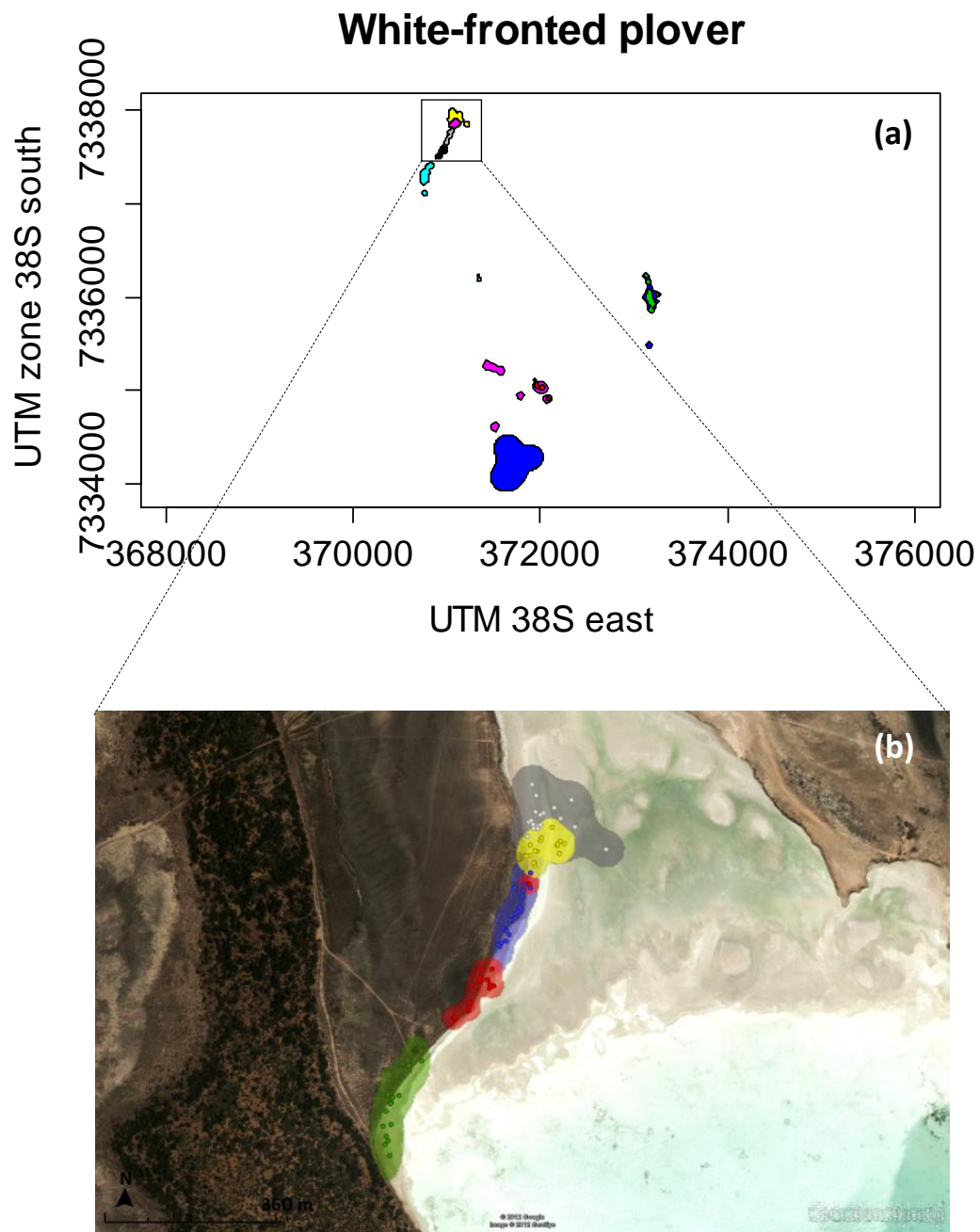
GLM were used to analyse mate searching area using Gaussian error structure and identity link function. Manipulation refers to whether a plover was kept in captivity or not. Days tracked refers to the number of days that a plover was tracked in the field. N<sup>o</sup> relocations refer to the number of relocations taken when searching for a mate.



**Figure 4.** Utilization distribution overlap index (UDOI), which estimate the amount of overlap between a plover and the UD of a sample population using the same space, in two plover species. The data are Box-Cox transformed and normalized using the parameter  $\lambda$ . The lower and upper borders of the boxes are lower and upper quartiles, respectively, the horizontal bar is the median and whiskers represent the lowest and highest observations. Circles denote outliers that are between the first and third interquartile from the nearer edge of the box.



**Figure 5.** (a) Polygons represent the 95% Kernel utilization distributions for each Kittlitz's plover (colours) sampled in the population. (b) Mate searching areas in Kittlitz's plovers as illustrated by 2 males (green and blue) and 2 females (purple and yellow) in Andavadaoka, Madagascar. Plovers overlapped their breeding sites. Each colour represents a single plover searching for a mate; points represent relocations and polygons represent 95% Kernel utilization distributions. Fieldwork was carried out between early February and late May 2010.



**Figure 6.** (a) Polygons represent the 95% Kernel utilization distributions for each white-fronted plover (colours) sampled in the population. (b) Mate searching areas of white-fronted plovers as illustrated by 3 males (green, red and blue) and 2 females (grey and yellow) in the north part of Lac Tsimanampetsosa National park, Madagascar. Plovers overlapped at the edge of their mate searching areas. Each colour represents a single plover searching for a mate; points represent relocations and polygons represent 95% Kernel utilization distributions. Fieldwork was carried out between early April and late June 2011.

## Discussion

Spatial movements and social interactions vary between closely-related species of plovers when searching for a mate. First, mate searching effort (area and distance moved) were smaller for the biparental white-fronted plovers than for the uniparental Kittlitz's plovers; the former moved shorter distance from previous breeding territories before settling and remating than the latter. In white-fronted plover, both sexes showed similar search effort before remating and tended to retain breeding territories waiting for a new mate. In addition, overlapping home range was limited with the larger population in this species, probably as both sexes tended to defend breeding territories against plovers of the same sex and from already mated pairs. These results suggest that in biparental and social monogamous species, such the white-fronted plover, restricted movements is a consequence of limited breeding vacancies. In other populations, the white-fronted plover has been reported to exhibit high breeding site-fidelity and territory retention within and between years (Lloyd 2008). Thus, competition for breeding vacancies should be strong restricting mating opportunities for both males and females. This was illustrated by the unmated white-fronted plovers which remated as soon as their formal mates were removed from their territories, probably to not lose their breeding status (Parra et al. 2014). Therefore, mate searching effort should be high for the floater population of single white-fronted plovers.

In contrast, female Kittlitz's plovers used larger areas and travelled longer distance than males to find a mate. Male Kittlitz's plovers tended to stay in their previous nest site, although the home ranges of both sexes were found to overlap with several other conspecifics in the population. These movements occur because Kittlitz's plovers exhibit a complex gregarious social behaviour where individual plovers join to flock for feeding and resting on grasslands and shore-lines; even members of breeding pairs join flocks due to males and females incubate in shifts through day and night (Urban et al. 1986; Hockey et al. 2005). In addition, breeding sites do not seem to be limited in this uniparental species because nests are at higher densities (10 – 30 metres apart) on grassland (Urban et al. 1986; Hockey et al. 2005).

Female Kittlitz's plover probably invest more in mate searching because it was found that males remated quickly than females, which means that males have



more mating opportunities than females (Parra et al. 2014). In our Kittlitz's plover population, we also found that males are the caring sex of plover chicks which might compensate for the mate searching effort incurred by females. Patterns of female-biased mate searching behaviour have been predicted in high density conditions where sperm is not a limited resource (Kokko & Wong 2007). For example, female fiddler crabs increase their mate searching effort in areas with high male-burrow densities allowing them not only to explore several males but also to escape from potential predators decreasing the costs of mate searching (DeRivera & Vehrencamp 2001; DeRivera et al. 2003). In addition, female condition can influence a mate searching behaviour; that happen if energy stores are high such as in pronghorn females (*Antilocarpa americana*), where weather conditions influence on females state, and in consequence on mate searching behaviour (Byers et al. 2006).

Finally, social encounters were limited between male and female white-fronted plovers, whereas social encounters between the sexes occurred frequently for male and female Kittlitz's plovers. As mentioned, Kittlitz's plover is a gregarious species with uniparental care pattern which make them to interact frequently between members of the population for feeding, anti-predatory behaviour and other social interactions such as finding a mate. These common encounter-sites might be the areas for searching and attracting receptive mates within food patches. Thus, the costs of mate searching should be low for females due to reduced risk of predation within flocks of plovers and energy costs associated with searching. As a consequence, females should spend more time to choose a male.

In contrast, the white-fronted plover is less social when searching for a mate. Males and females had few interactions with other plovers until they found a mate. If the costs of searching are related to defending a nesting site, males and females should stay together to protect a territory and spend less time searching for a mate to reduce the risk of losing both their nest site and breeding status. For example, patterns of restricted search are probably a consequence of competition with conspecifics for nest sites in female pied flycatchers (*Ficedula hypoleuca*); they might run a great risk of losing their breeding status if they leave a male nesting territory in an attempt to find a new mate and nest site (Slagsvold et al. 1988). In addition, in long-lived animals, such as the white-fronted plover (Lloyd 2008), high mate-fidelity between years reduces the search costs for females as they already

know the quality of their mates (Uy et al. 2000). In a previous study we found that remated individuals of white-fronted plovers reunited with their formal mates when release them from captivity (Parra et al. 2014); old established pairs may better improving their fitness benefits in terms of synchronization of breeding activities and reproductive success than new established pairs (Bried et al. 2003; Griggio & Hoi 2011).

In conclusion, we found different mate searching strategies in closely related plover species that exhibit different breeding systems. These findings have important implications to understand the role of mating opportunities on mate searching patterns. On the one hand, limited breeding vacancies and territoriality are probably important factors in influencing mating opportunities for male and females, and in turn, the mate searching behaviours of the sexes, since territoriality increase the degree of competition for limited resources such as nesting sites. Thus, the high costs of finding nest sites is probably an explanation of restricted searching movements and access to conspecifics such as in white-fronted plover which exhibit high mate and breeding site fidelity. In consequence, monogamy and biparental care are probably the best strategy to maximize reproductive success as revealed in other shorebird species (Lessells 1984; Gratto et al. 1985). On the other hand, in the Kittlitz's plover which exhibits variable breeding system, male-biased mating opportunities seems to have a direct effect on female-biased mate searching behaviour. The costs associated with mate searching behaviour, such as resources distribution, predation risk and foraging, seem to be low by the gregarious behaviour of the species and food abundance in the area (personal observations), facilitating a polygamous and uniparental care strategy in this species. Further studies should investigate the influence of predation and resource distribution on mate searching. In addition, it will be interesting to carry out demographic studies to understand the influence of sex ratios on mating opportunities and the direction and intensity of competition in males and females for mates and breeding territories.

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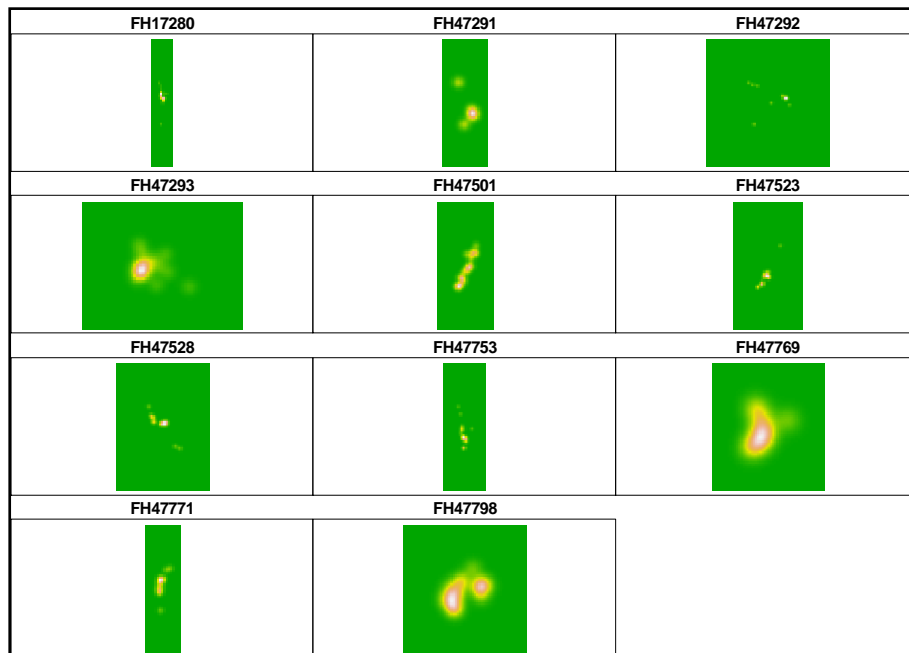
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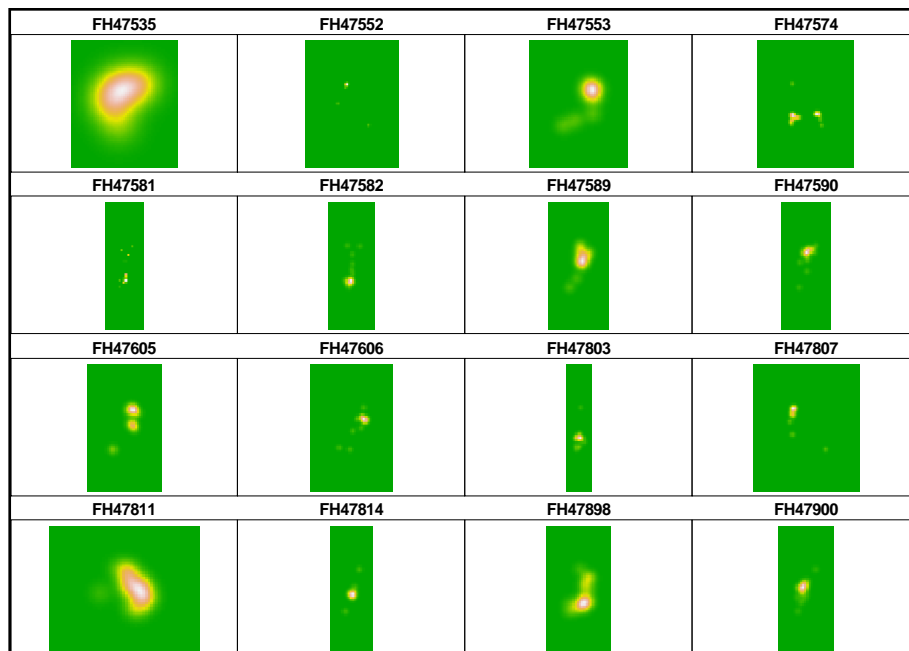
**Appendix****Table 1A.** Attributes of the two species plovers tracked by handle GPS in the south-west Madagascar in 2010 and 2011.

Species	Sex	No plovers	Mean locations	GPS SD locations	GPS Mean tracked	days SD tracked
Kittlitz's plover	F	11	16.55	7.16	28.91	12.49
	M	13	18.38	5.49	31.00	11.53
white-fronted plover	F	13	11.69	5.09	12.62	7.73
	M	12	13.58	5.40	10.17	8.18

(a)



(b)



**Figure A1.** Representation of utilization distributions when mate searching in the white-fronted plover (a) and Kittlitz's plover (b). That is the probability distribution of a plover using the space. The probability to find a plover increases from green to white colours.



## CHAPTER FIVE

### **Age and Gender Influence Camouflage in Sexually Monomorphic Plover Chicks**

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*Manuscript*

*Author's contributions*

**JEP:** study design, data collection, statistical analysis, manuscript preparation

**PG:** data collection in the lab

**RK:** manuscript improvement

**MB:** data collection in the field

**SZ:** data collection in the field

**AK:** statistical analysis, manuscript improvement

**TSz:** study design, manuscript improvement

**Abstract**

Predation is often a major selective pressure, and to evade predators various animals have evolved camouflage. Camouflage includes all forms of concealment such as cryptic colouration and behavioural responses that prevent detection. Camouflage is especially important for young animals with reduced mobility skills to evade predatory attacks. Here, we investigate chick camouflage, background matching, in two small shorebird species, white-fronted plover (*Charadrius marginatus*) and Kittlitz's plover (*C. pecuarius*). These plovers are common breeders in Africa, and monomorphic so that adult males and females are alike. Plover chicks are precocial, and they rely on their cryptic plumage to avoid predators. We took digital photographs of hiding chicks in their natural habitat, and then used a visual predator (*Homo sapiens*) to identify the chick on the photos. Using human observers, we test three hypotheses: detection time depends on (i) microhabitat choice of the chicks, (ii) chick age and (iii) chick sex. First we show that chicks appear to choose non-random positions when hiding from predators in both species of plovers. Second, we found that old chicks were more difficult to detect than young chicks. Finally, we found a sex difference in detection time in white-fronted plover chicks but not in Kittlitz's plover chicks. These results suggest that microhabitat selection, chick age and chick sex may all influence detection time, and thus mortality of chicks from predation in ground nesting birds where chicks rely on crypsis to evade predators.

**Keywords:** camouflage, cryptic coloration, experiment, predation, shorebirds

## Introduction

Predation is a strong and prevailing selection pressure in many animals. Camouflage and crypsis are some of the best studied examples of how potential prey may evade predators (Endler 1978; Stevens 2007; Stevens & Merilaita 2011). Concealment by blending into the environment, and disrupting the shape and contour of the animals (i.e. disruptive coloration) and associated behaviours (e.g. choosing appropriate microhabitats, hiding motionless) are especially important for young animals that are not yet fully capable evading the predators in other ways, e. g. flee, deter or fight off the enemies (Geoffrey & McGraw 2006; Stevens 2007). Thus offspring of many terrestrial animals match extremely well their environment providing some of the early support for Darwinian arguments of natural selection (Endler 1978; Stevens 2007).

One of the striking examples of camouflage is the plumage pattern of shorebird chicks (Colwell et al. 2007). Nearly all shorebirds (sandpipers, plovers and allies) nest on the ground, and the freshly hatched chicks rely on hiding and their cryptic plumage to escape predation. Predation is usually the main source of chick mortality since up to 90% of chicks can be predated before they fledge in shorebirds (Ricklefs 1969; Flint et al. 1995; Loegering & Fraser 1995; Colwell et al. 2005). Since visually searching predators (for instance harriers, falcons, shrikes and crows) are among the most significant causes of chick mortality, adopting cryptic plumage and hiding behaviour are imperative.

Although the patterning and colouring of downy plumage differ between shorebird species (Jehl 1968), the match between a hiding chick and the environment is striking (Figure 1). Chick may increase blending in the environment in three ways. First, once a predator is nearby, the chick can choose a microhabitat that hides part of their body from the predator, for instance they may hide next to an object (e.g. plant, stone or debris). Second, the chicks' downy plumage may match the colour of the substrate, so that light colours may predominate in downy plumage in chicks that grow up on light substrate, whereas chicks that live in dark substrate may exhibit darker plumage (Bergman 1955). Third, the spots and zigzags on dorsal side of the chick may disrupt the shape of their body helping their body contour to blend into the environment (Stevens 2007).

Whilst camouflage of eggs or chicks is often cited as a means to evade predators in ground nesting birds, the experimental evidence is scant (Stevens 2007; but see Lovell et al. 2013). Here we investigate chick camouflage in two ground-nesting shorebirds, the white-fronted plover (*Charadrius marginatus*) and the Kittlitz's plover (*C. pecuarius*). These small shorebirds (adult body mass approx. 35-37 g) are common breeding species in much of sub-Saharan Africa including Madagascar where we carried out the study (Zefania et al. 2010). Plover chicks are precocial i.e. leave the nest a few hours after they hatch, and are attended (but not fed) by the parents (Safford & Hawkins 2013). When predators approach the brood, the parents perform distraction displays such as injury-feigning to predators, while the chicks hide and remain frozen on the ground until the predator leaves the area (Urban et al. 1986; Hockey et al. 2005). Chick predators in Madagascar include kelp gull *Larus dominicus*, Caspian tern *Sterna caspia*, Madagascar kestrel *Falco newtoni*, Eleonora's falcon *Falco eleonora*, Sooty falcon *Falco concolor* and pied crow *Corvus albus* (Safford & Hawkins 2013). We took a photo of hiding chicks using a standardized setup (Fig 1), and then used a visual predator (*Homo sapiens*) to relocate the chick on the photos. Human observers have been used previously in studies of animal camouflage to mimic detection by visual predators (Glanville & Allen 1997, Fraser et al. 2007, Karpestam et al. 2013).

We have three hypotheses. First, we hypothesized that microhabitat selection influences camouflage, so that detection time will be longer in natural position of the chick than in a random position nearby. Second, we hypothesized that old chicks will be detected sooner than young ones, since older chicks are larger than young ones, so that large chicks may be more conspicuous than small ones (Cuadrado et al. 2001). Third, since chick mortality is sex-biased in a closely related species, the Kentish plover (*Charadrius alexandrinus*, Székely et al. 2004), we hypothesized that detection time of male and female chicks will be different. Support for the latter hypothesis would be surprising, since both white-fronted plovers and Kittlitz's plovers are sexually monomorphic: male and female plovers look identical both as chicks and adults, and sizes of male and female chicks are not different (see below).

## Materials and methods

### *Study species and study sites*

Fieldwork was carried out between 1st April and 23rd June 2011 at Lake Tsimanampetsotsa National Park (24° 3' S, 43°44' E) and wetland systems around the city of Toliara (23° 24' S, 43° 43' E). In both sites the plovers nest on the edges of salt marsh or sandy shores, and parents take their chicks around the nest sites for about four weeks when the chicks fledge (Zefania et al. 2010). Three observers worked together using a car to simulate a predation event. When a family was spotted approximately 70 – 100 m away using spotting scopes and binoculars, two observers kept watching the chicks by each observer focusing on a different chick (modal brood size is two chicks at hatching of the eggs, Safford & Hawkins 2013). The third observer ran directly to the family that provoked alarm calls by parent(s) which resulted in the chicks lying motionless on their chosen background (Figure 1a). The hiding chicks were then located by carefully searching the area by one of the observers using the instructions of the other two observers. Before picking up the chicks, digital photographs were taken in their natural hiding position using natural light and a Nikon Coolpix P80 camera mounted on 50 cm tripod. A Kodak grey card with a ruler and an identification badge were placed next to the chicks (Figure 1a). Then the chick was picked up, placed on a Kodak grey card and digital photographs were taken for a follow-up study (Figure 1b).

### *Molecular sexing and estimating chick age*

In total, we captured and photographed 28 Kittlitz's plover chicks and 8 white-fronted plover chicks. Morphological traits (body mass, tarsus length, wing length and bill length) were measured with sliding calliper, wing ruler, and spring balance (see details in Kentish plover field guide, [http://www.bath.ac.uk/biosci/biodiversity-lab/pdfs/KP\\_Field\\_Guide\\_v3.pdf](http://www.bath.ac.uk/biosci/biodiversity-lab/pdfs/KP_Field_Guide_v3.pdf)). All chicks were ringed with an individual combination of numbered SAFRING metal bands from the University of Cape Town, South Africa. The age of the chicks were as follows (mean  $\pm$  SD age in days: male Kittlitz's plover: 15.14 days  $\pm$  11.55,  $N = 14$ ; female: 17.8 days  $\pm$  11.52,  $N = 14$ ; male white-fronted plover: 3.6 days  $\pm$  3.15,  $N = 4$ ; female: 7.31 days  $\pm$  3.44,  $N = 4$ ). The age of chicks with unknown hatch date was estimated using tarsus length of known aged chicks, since tarsus growth is linear in the closely related Kentish plover

(Szekely & Cuthill 1999). 53 and 28 tarsus measurements were taken from 28 and 13 Kittlitz's plovers and white-fronted plovers, respectively (Kittlitz's plover: age (in days) =  $2.79 * \text{tarsus (in mm)} - 54.77$ ,  $r^2 = 0.93$ ; white-fronted plover age (in days) =  $3.15 * \text{tarsus (in mm)} - 57.13$ ,  $r^2 = 0.93$ ). As mentioned before, size of male and female chicks has no indication of any difference as chick age (interaction sex by age of chicks on tarsus length ANCOVA Kittlitz's plover:  $t = 1.717$ ,  $P = 0.0871$ ; ANCOVA white-fronted plover:  $t = -0.537$ ,  $P = 0.593$ ).

Sex of plover chicks was determined by molecular sex-typing (dos Remedios et al. 2010). A small blood sample was taken from the leg vein by puncturing, collecting drops of blood (~25  $\mu\text{l}$ ) in capillary tubes, and storing in Queen's Lysis Buffer. DNA was extracted from blood samples using the Ammonium Acetate extraction method (Miller et al. 1988; Richardson et al. 2001). For molecular sex-typing, Z- and W-chromosome specific genes were amplified via polymerase chain reaction (PCR) using the Z-002B/Z-002D primers (Dawson 2007). For additional certainty in sex assignment, the W-chromosome specific Calnex-31 primers, developed in the genus *Charadrius* were utilized (Küpper et al. 2006). PCR amplification was conducted on a DNA Engine Tetrad 2 Peltier Thermal Cycler under the following conditions: 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 56°C for 90 s, 72°C for 60 s with a final extension of 60°C for 30 min. Samples were visualized on an ABI 3730 automated sequencer. IR Dye-labelled tailed primers separated the products of Z-002B/Z-002D primers into either one (ZZ) or two bands (ZW), indicating male or female respectively. The W-specific Calnex-31 product appeared as one band indicating female only. Images were scored using GeneMapper software version 4.1 (Applied Biosystems). Molecular sexing was repeated a different sample set (11 Kittlitz's plover and 10 white-fronted plover individuals), and in all 21 cases sexing provided consistent results.

### *Image recognition*

We carried out an experiment at the University of Bath (UK) using students as subjects of the experiments. The objective of the experiment was to test whether chicks at their original location had different detection times from the ones at random location. In total, 18 photos of Kittlitz's plover and 7 photos of white-fronted plover were used in the experiment. From each original photograph (one photo for

each chick, 25 images in total), we created three new images by randomly choosing one out of eight rotations (0, 45, 90, 135, 180, 225, 270 and 315°) and one out of 9 positions using Photoshop CS5 (Fig. 2A - B) keeping the scale constant. Plover chicks were positioned avoiding areas that increase their contrast such as shadows by running a new randomization. Some photographs were excluded from the random set because chicks were difficult to place randomly on the background without increase contrast. This study included 21 participants from University of Bath. For each participant, 25 original photograph and three x 25 manipulated images were presented in a random order. At every 10 second one image was shown on a computer screen at the same distance of 65 cm from the participants. We also investigated whether detection time was different between species, sex and age of the two plover chick species. All the photographs were at different age classes (see chick range age above).

In the experiment, E-Prime® 2.0 was used to measure the time elapsed to detect a chick on a digital image. If a participant detected a chick on the screen, he/she was required to press the space bar of a keyboard (detection time in milliseconds). To confirm whether the participant indeed detected the chick, then the same image was displayed again and the participants were asked to click the location where the chick was detected using a PC mouse. Chick detection was scored as 'success' when a chick was detected and its location was correct, and 'failure' if the chick was not detected within 10 seconds or if the participant did not point to the correct location of the chick on the screen. For failed records when the observer failed to spot the chick for 10 second the maximum time was assigned (10 seconds), whereas for records when the observer pointed to the wrong location the time of misdetection was assigned.

The experiment was approved by the Ethics Committee of the Department of Psychology at University of Bath. 21 human subjects were recruited from University of Bath. All subjects were required to have normal vision, or corrected to normal vision by glasses or contact lenses. Participation was voluntary, and participants were free to withdraw at any point during the experiment. All volunteers provided their written consent to participate in the experiments, and received £5 compensation for their time.

*Statistical Analyses*

Mixed effects Cox model fit by maximum likelihood was used to compare detectability rates of chicks between random and original position (fixed factor). In addition, the models included species and chick sex as factors, and chick age as a covariate (fixed terms). There was a significant 4 way interaction between the fixed variables (factors and covariate, see Results), so we analysed the two species separately. For the latter 2 models, three way interactions (position x age x sex), and all two-way interactions were included between fixed variables. The random effects, subject-ID and photo-ID, had a significant effect on the fit of the models when comparing with a model containing only one of the random effects (Kittlitz's plover models: Likelihood ratio tests (LRTs) subject-ID:  $\chi^2_1 = 127.28$ ,  $df = 1$ ,  $P < 0.001$ ; photo-ID:  $\chi^2_1 = 87.09$ ,  $df = 1$ ,  $P < 0.001$ . White-fronted plover models: Likelihood ratio tests (LRTs) subject-ID:  $\chi^2_1 = 14.72$ ,  $df = 1$ ,  $P = 0.0001$ ; photo-ID:  $\chi^2_1 = 25.49$ ,  $df = 1$ ,  $P < 0.001$ ). Thus, both random effects were also included in the models.

The terminal event (outcome) in both models was the detection of chicks. Those chicks that were not detected within 10 seconds, or not detected correctly, were treated as censored. For each species we analysed the 25 original images against each of three sets of 25 manipulated images separately. The three analyses revealed qualitatively the same results, except in one case where the interaction of position by sex was not significant (Appendix 1). Therefore, we report the result of one dataset. We would like to point out that chick detection experiments did not test apart the effect of background using a control treatment: only-background (see methods). Therefore, conclusions must be taken with caution. All analyses were carried out in R package using 'coxme' (R Development Core Team 2010, Therneau 2012).

**Results**

There was a significant 4-way interaction between species, position, chick sex and chick age, and therefore, we analysed the data separately for white-fronted plovers and Kittlitz's plovers (Table 1).

For white-fronted plovers, a significant 3-way interaction indicated that the effect of chick sex on detection time depended on its age and position (Table 1). In



their original position the 6 day and 11 day old white-fronted male chicks had the longest detection time, in contrast with female chicks (Fig 3A, Table 1). In their random position neither sex nor age predicted detection time (Fig 3B, Table 1; Table 2 show parameter estimates after removal of the non-significant interaction term).

In white-fronted plover chicks, detection time of males increased over age in original position, but less so in random position as indicated by significant age by position interaction (Fig 3C, Table 1). There was a similar, albeit somehow weaker effect in female white-fronted chicks (Fig 3D, Table 1).

For Kittlitz's plover, the 3-way interaction between sex, chick age and position had no effect on detection time (Table 1). In their original position, detection time tended to increase with chick age and this trend was consistent more for male than female chicks in plover chicks 10 days old (Figure 4A), however this interaction between age by position in each sex had no statistical effect on detection time. In their random position neither sex nor age predicted detection time as well (Fig 4B, Table 2). In Kittlitz's plover, detection time increased over age in the original position, but less so in random position as indicated by significant position by age interaction both in male chicks and female chicks (Table 1, Fig 4C and D).

**Table 1.** Likelihood ratio tests for interactions on plover chick detection time of the white-fronted plover and Kittlitz's plover.  $\chi^2$  and p values in brackets are given (df = 1 in all tests). Bold type indicates significant effects.

Interaction	Data			
	both species			
Species*position*sex*age	5.46 ( <b>0.020</b> )			
	white-fronted plover		Kittlitz's plover	
Sex*age*position	6.50, ( <b>0.011</b> )		0.01, (0.933)	
	original	Random	original	random
Sex*age	7.78, ( <b>0.005</b> )	0.34, (0.558)	0.08, (0.782)	0.96, (0.327)
	females	Males	females	males
Position*age	10.89, ( <b>0.001</b> )	31.70, (< <b>0.001</b> )	5.16, ( <b>0.023</b> )	5.81, ( <b>0.016</b> )

**Table 2.** Parameter estimates for sex and age after removal of the non-significant interaction terms from the models in Table 1.

Parameter	White-fronted plover		Kittlitz's plover			
	random position		original position		random position	
	B±SE	P	B±SE	P	B±SE	P
Sex	-0.38±0.647	0.558	-0.63±0.627	0.311	-0.09±0.454	0.839
Age	-0.08±0.083	0.315	-0.06±0.038	0.109	-0.02±0.028	0.511

**A.**

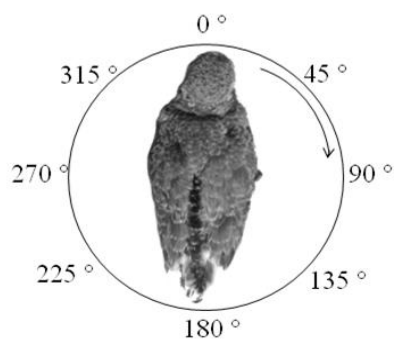


**B.**

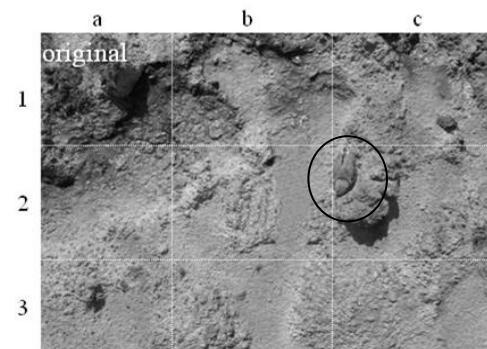


**Figure 1.** Digital photograph of young plovers took at natural background A. and on a Kodak grey card B.

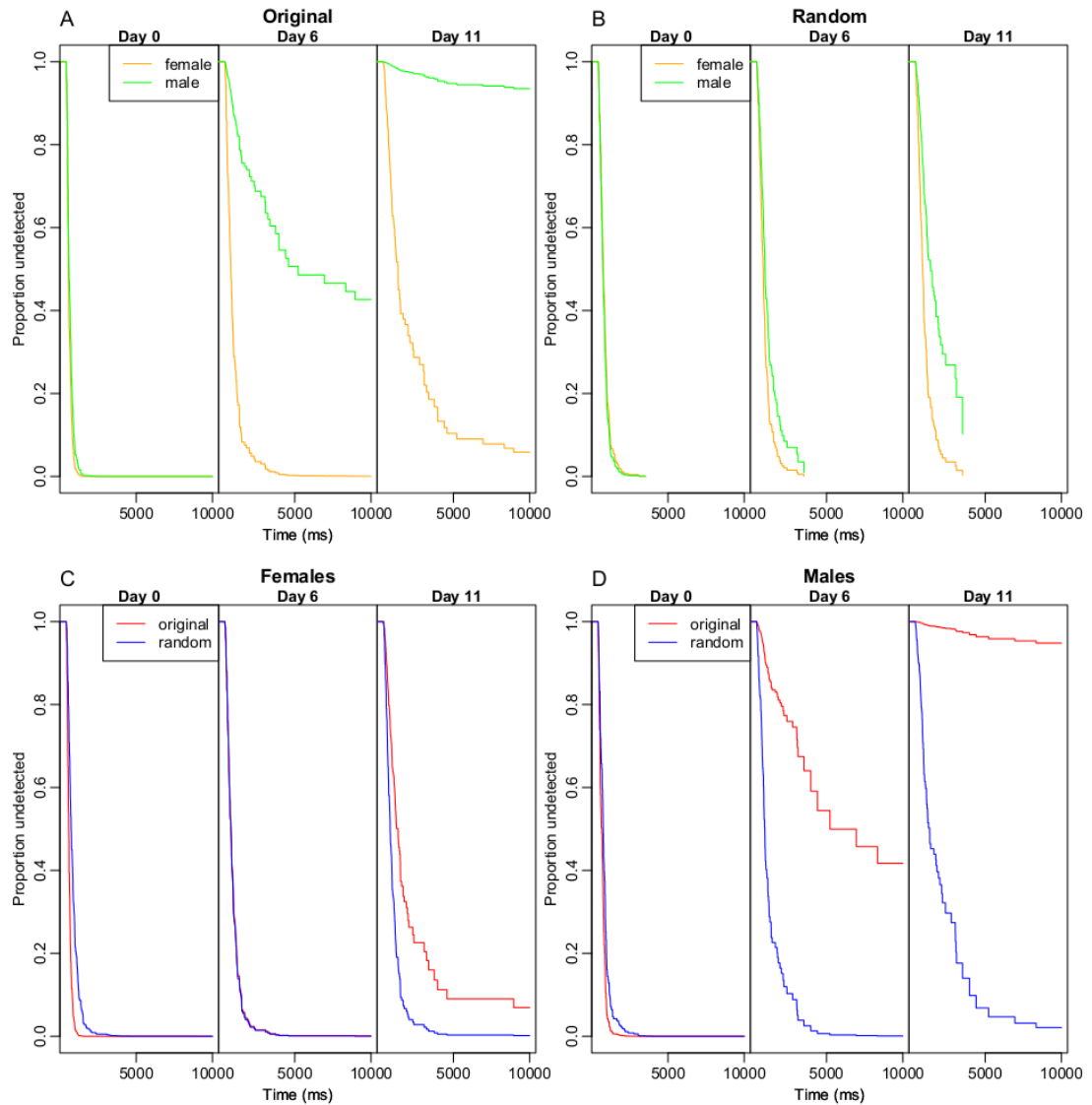
**A.**



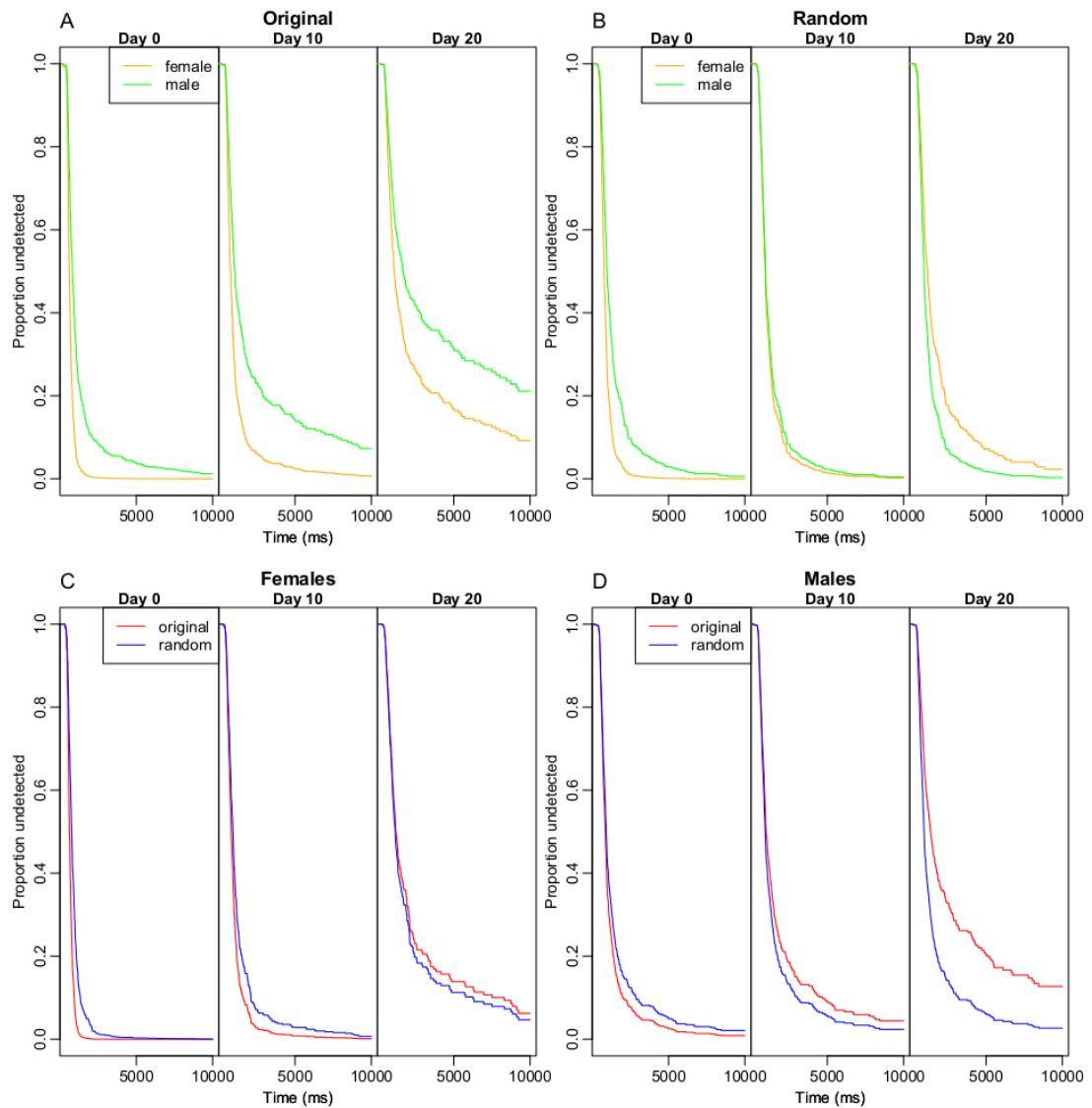
**B.**



**Figure 2.** Method to create images of A. chicks randomly oriented and B. chicks randomly positioned (details in methods section).



**Figure 3.** Survival curves of the probability that white-fronted plover chicks are undetected in ten second trials. A. Curves of the interaction between sex and age of plover chicks positioned on original backgrounds. B. Curves of the interaction between sex and age of plover chicks positioned on random backgrounds. C. Curves of the interaction between position on background and age of female plover chicks. D. Curves of the interaction between position on background and age of male plover chicks. Note that age was a continuous covariate in the model, however, for illustration purposes the predicted values are given only for three ages here. The curves are based on parameter estimates of the mixed effects Cox models.



**Figure 4.** Survival curves of the probability that Kittlitz's plover chicks are undetected in ten second trials. A. Curves of the interaction between sex and age of plover chicks positioned on original backgrounds. B. Curves of the interaction between sex and age of plover chicks positioned on random backgrounds. C. Curves of the interaction between position on background and age of female plover chicks. D. Curves of the interaction between position on background and age of male plover chicks. Note that age was a continuous covariate in the model, however, for illustration purposes the predicted values are given only for three ages here. The curves are based on parameter estimates of the mixed effects Cox models.

## Discussion

Our study provided three key results. First, we showed that chick position had significant influence on detection times, and chicks appear to choose non-random positions when hiding from predators in both species of plovers. This pattern is consistent with studies of moths that present non-random resting orientations on natural substrates that appear to improve their crypsis (Webster et al. 2009). Finding an appropriate microhabitat is especially challenging for the chicks, since they usually have only a few seconds to hide before the predators strike. This result should be treated with some caution as the no introduction of background-only element in the treatment could cause difference on chick detection times.

Some ground-nesting shorebird species nest in different habitats or patches, where different rates of predation occur (Colwell et al. 2011). Therefore, a trade-off in the degree of matching between different habitats might influence prey camouflage behaviour to avoid predators. For example, in Kentish plover natural grasslands produced more hatchlings than human-made fishponds (Székely 1992), likewise in the snowy plover, *Charadrius nivosus*, habitat was shown to have a significant influence on the reproductive success; the proportion of hatching and fledging young plovers was higher on river than beach habitats (Colwell et al. 2011). In addition, corvids were found more frequently on river habitats and were one of the main predators of chicks and eggs (Colwell et al. 2005, 2011). In addition, some colour patterns and behaviour traits of chicks may enhance chick survival in a specific habitat. For example, in a laboratory experiment Lovell et al. (2013) showed that female Japanese quails (*Coturnix japonica*) chose substrate that matches the coloration of their eggs, presumably to increase survival of their eggs. In the Caspian tern (*Hydroprogne caspia*), intermediate coloured downy chicks are suggested to resemble better the background of nesting sites than do extreme downy chick morphs (Chaniot 1970). In our study, chicks of both species were observed using different feeding grounds. In white-fronted plover, for example, the brood stays within their parents' territory until they fledge. It is probable that chick colour patterns are locally adapted to the surrounding sandy habitat to reduce predator attacks. In contrast, Kittlitz's plover parents lose their territorial status soon after chicks hatch and broods wander around different feeding grounds. Therefore, chick colour patterns are probably more sensitive to be detected in some habitats than others.

Second, we found that old chicks were more difficult to detect than young chicks. This is in contrast with our expectation, and suggests that in spite of having larger body size, old chicks are better hiding than matching the background. As chick age, hiding behaviours probably improve with escape performance. Chicks acquire independence from the parents to thermoregulate, and then brooding vigilance; this should result in a development of their own vigilance and response to predators. The significance of this result is that behaviour and morphological traits improve as chicks age to avoid predators. This might be possible due to developmental changes, a learning process or simply by age-related traits under natural selection. Although, recognition of avian predators has been proved to be innate in young plovers, parentally-taught behaviour is suggested to be also an important factor to increase survivorship in chicks (Saunders et al. 2013). Another remarkable change in plover chicks is their plumage. Therefore, during the development from downy to first juvenile plumage might also change their camouflage behaviour strategies (Tree 1974; Urban et al. 1986; Hockey et al. 2005).

Third, we found a sex difference in detection times of white-fronted plovers but not in Kittlitz's plover. Although sample sizes were limited to 4 males and 4 females, male white-fronted chicks were more difficult to find than female white-fronted chicks. We suggest three explanations for this difference: (i) male and female chicks have different plumage pattern that makes males more difficult to detect; (ii) male and female downy plumage have different colouration and the overall colour of males match better the background; (ii) male chicks chose more concealed hiding place than females. The degree of sexual dimorphism might influence juvenile mortality, especially sexual size dimorphic species (Clutton-Brock et al. 1985). Further studies, then, are needed to test whether plover chicks have sex difference in plumage patterns and colouration.

In conclusion, using a combination of field work, molecular and experimental techniques, we found that two ground-nesting Madagascar plover chicks select non-random backgrounds for camouflage. Old chicks were more difficult to detect than young chicks which suggest that hiding behaviour improve with age. Male white-fronted plover chicks were more difficult to detect than female chicks, suggesting a differential survival much earlier than previously suspected and to be caused in part by differential predation on sexes. Contrary to our predictions, our data indicated that

in sexual size monomorphic species sex-biased mortalities probably occur and suggest that predation is one of the potential factors influencing juvenile sex ratios.

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**Appendix 1.** Likelihood ratio tests with the three different random datasets of the experiment.  $\chi^2$  and  $P$  values are given (df = 1 in all tests). Bold type indicates significant effects. WFP = white-fronted plover; KiP = Kittlitz's plover; M = male; F = female.

Model		Rand1		Rand2		Rand3
4way interaction		$\chi^2=5.455$ , $P =$ <b>0.020</b>		$\chi^2=6.733$ , $P =$ <b>0.009</b>		$\chi^2=0.182$ , $P =$ 0.670
WFP	3way	$\chi^2=6.503$ , $P =$ <b>0.011</b>		$\chi^2=12.249$ , $P <$ <b>0.001</b>		$\chi^2=0.870$ , $P =$ 0.351
WFP	Original	$\chi^2=7.779$ , $P =$ <b>0.005</b>		$\chi^2=7.779$ , $P =$ <b>0.005</b>		$\chi^2=7.779$ , $P =$ <b>0.005</b>
WFP	Random	$\chi^2=0.343$ , $P =$ 0.558		$\chi^2=0.003$ , $P =$ 0.956		$\chi^2=2.804$ , $P =$ 0.094
WFP	F	2way $\chi^2=10.886$ , $P =$ <b>0.001</b>		$\chi^2=6.924$ , $P =$ <b>0.009</b>		$\chi^2=26.675$ , $P <$ <b>0.001</b>
WFP	M	2way $\chi^2=31.699$ , $P <$ <b>0.001</b>		$\chi^2=49.590$ , $P <$ <b>0.001</b>		$\chi^2=27.953$ , $P <$ <b>0.001</b>
KiP	3way	$\chi^2=0.007$ , $P =$ 0.932		$\chi^2=10.769$ , $P =$ <b>0.001</b>		$\chi^2=22.159$ , $P <$ <b>0.001</b>
KiP	Original	2way $\chi^2=0.076$ , $P =$ 0.782		$\chi^2=0.076$ , $P =$ 0.782		$\chi^2=0.076$ , $P =$ 0.782
KiP	Random	2way $\chi^2=0.960$ , $P =$ 0.327		$\chi^2=4.807$ , $P =$ <b>0.028</b>		$\chi^2=6.129$ , $P =$ <b>0.013</b>
KiP	F	2way $\chi^2=5.164$ , $P =$ <b>0.023</b>		$\chi^2=2.019$ , $P =$ 0.155		$\chi^2=0.846$ , $P =$ 0.358
KiP	M	2way $\chi^2=5.810$ , $P =$ <b>0.016</b>		$\chi^2=41.349$ , $P <$ <b>0.001</b>		$\chi^2=32.588$ , $P <$ <b>0.001</b>

## CHAPTER SIX

### **Conclusions and future research: Breeding systems in response to variation in mating opportunities**

Jorge E. Parra




## General discussion

I argue in this dissertation that animal reproductive strategies depend on variation in the social environment in which animals live. That is, spatial and temporal variations in social structures influence the emergence of different reproductive strategies. Accordingly, I investigated the role of mating opportunities on the reproductive strategies of small plover species of genus *Charadrius*. I found that mating opportunities differed between closely-related species that exhibit differing mating and parental care patterns (chapter three). I show that mating opportunities influence male and female mating and parental care strategies such as brood attendance, courtship behaviour and mate searching behaviours (chapters two, three and four) (Table 1). Consequently, the trade-off between the benefits of providing care and the benefits of deserting the offspring and remating seems to be balanced by mating opportunities.

I also studied different contributory factors that have been proposed to influence variation in mating opportunities (Table 1). These included both ecological and social factors which may impact demographic properties of populations such as the adult sex ratios (ASR), and in turn, the operational sex ratios (OSR), i.e. ratios of sexually active males to females (Emlen & Oring 1977; Clutton-Brock & Parker 1992; Kvarnemo & Ahnesjö 1996; Donald 2007; Kokko & Jennions 2008). I found that pair bond stability had also implications on the number of available mates in a population (chapter three). Long-term pair bonds and territoriality may respond to limited reproductive resources such as breeding vacancies, influencing the number of available mates (chapter three and four). In addition, sex-specific mortality in different life-history stages may also impact on the ASR and OSR, and in turn, on mating opportunities (chapter five).

These results demonstrate that mating opportunities vary between closely related species with different mating and parental care patterns, and suggests that a demographic property of the population, such as the OSR might impacts on mating opportunities and predicts courtship behaviour and parental care consistently with theoretical models (chapter one). This supports the idea that variation in the social environment has a major influence on breeding systems evolution.

**Table 1.** Summary of mating and parental care aspects of the breeding system of three species of *Charadrius* plovers.

Species	Predation	Spatial and temporal distribution of resources		Mating strategies		Parental care strategies	Mating opportunities <sup>2</sup>	Photo
	Chick detectability <sup>1</sup>	Breeding vacancies <sup>2</sup>	Mate searching effort <sup>3</sup>	Mate fidelity <sup>2</sup>	Courtship displays <sup>2</sup>	Brood attendance <sup>4</sup>		
Kittlitz's plover male	high	high	moderate	low	moderate	high	high	
Kittlitz's plover female	high	high	high	low	high	low	low	
white-fronted plover male	moderate	low	high	high	high	high	low	
white-fronted plover female	high	low	high	high	moderate	high	low	
Madagascar plover male	?	?	?	?	?	high	?	
Madagascar plover female	?	?	?	?	?	high	?	

<sup>1</sup>Chapter five; <sup>2</sup>Chapter three; <sup>3</sup>Chapter four; <sup>4</sup>Chapter two

### **Mating opportunities in relation to ASR and OSR: follow up ideas**

One striking result was that the male-biased mating opportunities in Kittlitz's plover were the opposite of those found in the Kentish plover. This contrast may emerge in two ways. On the one hand, ASR should be female-biased in the Kittlitz's plover, and females may be more likely stay with and provide care for the brood than males; contrary to the Kentish plover that exhibits heavily male-biased ASR and male-biased parental care (Székely & Lessells 1993; Amat et al. 1999; Kosztolanyi et al. 2011). On the other hand, the male-biased mating opportunity may emerge in Kittlitz's plover, if males care more often for the brood than females creating a shortage of males in the population. Accordingly, I found that Kittlitz's plover brood attendance behaviour was male-biased (chapter two). These results might imply that male-biased mating opportunities emerge because male plovers provide care more frequently than females, and then, males probably become rare in the population. In consequence, it would be interesting to use demographic modelling to estimate variation in ASR between Malagasy plovers that may be associated with the mating opportunities estimated (Székely et al. 2014). Experimental manipulation of the ASR will be another approach to test whether males and females change their mating and parental care behaviour in response to changes in the ASR (male-biased, female-biased, unbiased ASR).

Life-history traits could be used to test whether stage-specific mortalities and maturation rates are related to ASR, and then, to mating and parental care modes. This is because, parental care is suggested to be favoured by high mortality rates and long duration in one or multiple stages, since offspring cannot survive without parental care (Klug & Bonsall 2010; Klug et al. 2012). Madagascar, white-fronted and Kittlitz's plovers exhibit biparental care during the incubation stage, this imply that eggs might have low chance of surviving in the absent of one or both parents. However, brood attendance behaviour differs between species which suggest that chick mortality is comparable between uniparental and biparental brood care strategies. That is, for Kittlitz's plover one parent is enough to rear the brood whereas in Madagascar and white-fronted plovers both parents are needed. To test this prediction, I propose to compare chick mortalities between species with different breeding systems.

Ecological factors such as resources necessary to mate and care may also influence in sex roles over reproduction. Variation in breeding site abundance appears to affect the ASR and the direction of sexual selection (Forsgren et al. 1996). In the strawberry dart-poison frog (*Dendrobates pumilio*), for example, female-only care and high nest site availability was related to a female-biased ASR and low male-male competition (Prohl 2002). As mentioned, breeding vacancies are probably limited resource for biparental species, and a strong competition for nesting territories may facilitate high pair bond stability and site fidelity (chapter three). Thus, I propose to experimentally manipulate the occupancy of breeding territories and test how these may influence mating systems through competition for critical resources for breeding.

Finally, I argue that the experimental approach used to quantify mating opportunities in the small plover species is one of the most reliable forms to know difference in mating opportunities between sexually active males and females. A comparable study carried out in two spotted gobies *Gobiusculus flavescens* (Forsgren et al. 2004) showed that changes in courtship behaviour over the breeding season coincided with a shift in OSR, and in turn mating opportunities, that was male-biased early in the season and female-biased toward the end of the breeding season. My work goes beyond in two respects. First, we investigated three closely-related species and found differences both in their mating times and courtship behaviour. Second, we experimentally assessed mating opportunities using mating times of adults in full breeding condition, whereas based their OSR estimates on sampling males and females in their study area. We argue that experimental assessment of mating opportunities is a direct and more reliable indicator of mating prospects for males and females than sampling adults and classifying whether they appear to be in breeding condition or not. Therefore, experimental assessment of mating opportunities between populations at different geographical and temporal scales can provide insights into the breeding behaviour of the species.

### **Final remarks**

In this thesis, I empirically tested current theoretical models about the evolution of breeding systems. I showed that mating opportunity is a key component of the social environment of animal populations, since it can modulate mating and parental care



## CONCLUSIONS

efforts. I suggest that ASR has a major influence on mate availability and then reproductive strategies. Thus, empirical studies of the relationship of ASR and breeding systems can support recent comparative studies that suggest that sex roles are associated with skewed adult sex ratios (Liker et al. 2013, 2014). It is also important to understand the causes of ASR variation, since differential sex and stage-specific mortalities and maturation rates have been suggested to be important parameters to estimate ASR (Donald 2007b; Kosztolanyi et al. 2011). The implications of skewed ASR and mating opportunities should give us interesting insights into, for example, the causes of extrapair paternity that have been suggested to influence pattern of parental care (Møller 2000). In addition, it would be interesting to investigate whether mate choice will favour an increase in paternal care when ASR and mating opportunities skewed toward males. This is because, females of some species choose males based on their parental skills to increase offspring fitness (Lindström et al. 2006; Alonzo 2010; Klug et al. 2012).

The diversity of breeding systems of shorebirds (sandpipers, gulls, plovers and allies) offers a great opportunity to investigate how different mating and parental care strategies evolve. This group has been suggested to exhibit similar breeding systems to ancestral birds where paternal care seems to be the earliest form of care (Wesolowski 2004; Varricchio et al. 2008). Therefore, studying the evolution of breeding systems in shorebirds may reveal how monogamy and polygamy have emerged in birds since the split from dinosaurs.

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## CONCLUSIONS

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## APPENDIX ONE

# **Behaviour and ecology of breeding plovers in Andavadoaka, South-west Madagascar**

Fieldwork Report

6 February – 13 May 2010

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## Abstract

Fieldwork was carried out between 6 of February and 13 of May in Andavadoaka region, South-west of Madagascar. The main objective was to implement an experiment of the mating opportunities of the Kittlitz's plover in the area. The background of the experiment was to test hypotheses of the breeding system evolution through operational sex ratio; the ratio of sexually active males to females. It was also studied the breeding ecology of two other species of plovers: Madagascar plover and White-fronted plover. Finally, an ornithological survey was conducted to establish the importance of the Andavadoaka region for conservation. The experimental methodology was fit in the area using 36 individuals of Kittlitz's plover, 18 males and 18 females, for measuring ornamentation, movements and behaviours. Preliminary results revealed that, males stayed nearer to their previous territory, used smaller areas of action and re-mated more quickly than females. These novel results open questions about the causes of the breeding behaviour of this population. However, we need to analyse all data to know whether these trends remain (molecular sexing of all plovers). Andavadoaka has also a great population of Madagascar plover and White-fronted plover to study and monitoring the breeding ecology of these species. Finally, the area is of special biological interest due to the occurrences of many resident and migratory water and terrestrial birds. Four vulnerable species and one near threatened were recorded: Long-tailed Ground-roller (*Uratelornis chimaera*), Subdesert Mesite (*Monias benschi*), Humblot's heron (*Ardea humbloti*), Madagascar plover (*Charadrius thoracicus*), and Reunion harrier (*Circus maillardi*). The Andavadoaka region meet several RAMSAR criteria (The Convention on Wetlands of International Importance), which must be studied in depth to inform conservation and wise use of this area.

## Background

Sexual conflict is defined as the different interest of male and female over reproduction which gives benefits for one sex and costs for the other on fitness. Conflict between sexes occurs because each one spends different time and energy allocating resources to mate attraction and mating, and / or caring the offspring (Chapman et al., 2003, Parker, 2006). These two ways of conflicts, over mating behaviour and parental care, influence the diversity of breeding systems among organisms through a trade-off between sexes (Alonzo, 2010, Reynolds, 1996).

Conflict over parental care is one of the factors to explain breeding systems, and several hypotheses have been proposed to explain the benefits of care or desert the brood. On the one hand, caring the offspring by the couple should favour the survivorship of the offspring decreasing nest predation (Halupka and Greeney, 2009, Smith, 1977), or/and uniparental care expend more energy than biparental care (Brunton, 1988b, Brunton, 1988a, Smith, 1977). On the other hand, deserting the offspring should increase the mating opportunities or enhance adult survival (Smith, 1977, Szekely et al., 1999).

The impact of mating opportunities on sexual conflict might be understood through operational sex ratio which is the proportion of fertile females to adult males (OSR, (Emlen and Oring, 1977). To investigate whether different breeding strategies have been originated according to OSR, it is necessary to know the mating opportunities for males and females in a given time. Szekely *et al.* (1999) developed an experimental approach with the Kentish plover in Turkey, where was used the re-mating time spending by each sex in finding a new mate, as a direct measure of mating opportunities. This experiment found that males spend more time finding new mates than females. As consequence, there appears a male-biased care relates to female-biased mating opportunities in this population (Szekely, 2006). Hence, some predictions emerging from sexual conflict related to OSR. 1) As mating opportunities decrease in a given population, more time is predicted to be necessary for both sexes in finding new mates. Therefore, cooperative behaviour such as biparental care is the best strategy to increase the fitness of the male and female. 2) As mating opportunities increase, more conflict between sexes and uniparental care is predicted to be the best strategy to increase the fitness of one sex. In the latter

case, either the male or female could have more opportunities to re-mate depending on potential rate of reproduction.

Shorebirds are an excellent ecological model species to study sexual conflict due to their diverse breeding systems from social and genetic monogamy to polyandry and polygyny and from biparental care to uniparental care (Reynolds and Szekely, 1997). To study the breeding system evolution in shorebirds through sexual conflict, it has been analysed several hypotheses related with their sexual size dimorphism, demanding of young, migratory behaviour and mating opportunities (Garcia-Pena et al., 2009, Owens, 2002, Reynolds and Szekely, 1997, Szekely, 2006, Szekely et al., 2006, Thomas and Szekely, 2005, McNamara et al., 2000). All this massive work with shorebirds however is lacking of more empirical work to test the models and correlation studies on sexual conflict.

The objective of this fieldwork season was:

1. To investigate the mating opportunities and its benefits in males and females of the Kittlitz's plover *Charadrius pecuarius*.
2. To study the breeding ecology of Madagascar plover, White-fronted plover and Kittlitz's plover.
3. To establish the ornithological importance of the Andavadoaka area.

## Methods

The fieldwork was carried out in Andavadoaka region (22° 02' 08" S, 43° 39' 08" E), South-west coast of Madagascar (Fig. 1), from February 16<sup>th</sup> until April 27<sup>th</sup> 2010. The area consists of different habitats of costal euphorbia forest, spiny forest, mangrove, coasts, and several salt-marshes where the Kittlitz's plover was found.

Methods were similar to Szekely et al., (1999), and field methods followed the Kentish plover guide (Szekely et al., 2008). 18 pairs of Kittlitz's plover were caught between February and April. Morphological traits (body mass, tarsus length, wing length and bill length) were measured for each individual. It was also taken a blood sample and ornamentation pictures from each individual following the practical guide to photograph ornaments of plovers (Argüelles-Ticó 2008). Additionally, each individual were ringed with an individual combination of colour

rings and SAFRING metal ring. One parent (male or female) was released randomly before its flanks were dyed with green marker. The other parent was taken into an aviary (eight cages 1m height x 1.5 length x 1.5 width each in Coco Beach Hotel near to the beach), and only released after their mate had found a new mate. Kittlitz's plovers are sexually monomorphic then at the time of the manipulation we did not know the sex of the released plovers. The clutch of eggs was distributed into other plover nests. They were checked every 4 days in their new nests.

Eggs were measured, and the number of days that eggs had been incubated was estimated. The released plovers were searched for every day, and when found; a behavioural sample of every 30 seconds was recorded by 30 minutes for 2 observers. The re-mated individuals were identified by courtship, nest-scraps or copulation. New pairs were checked for clutches every day, and their nest were followed until hatching. The behaviour of parents was attempted to record two times both before and after they found a new mate.

Captive plovers were fed with appropriate food and drinks twice per day every day to maintain their good health using a high protein meals, blue mawseed, bird food supplement, hempseed, honey, dried flies and pinhead oatmeal (Prosecto). They were also supplied with live beetles captures in transect of 10 pit fall traps in their feeding campus on the salt-marshes. Twice a day the beetles were collected from the traps as a food supply to the captivity plovers. They were released after their former mate found a new partner. Before release, each plover was measured and marked by green marker. The released individuals were searched every day following the same methodology as above.

Additionally, a student of the University of Toliara was trained for studying the breeding ecology of Madagascar plover (*Charadrius thoracicus*), White-fronted plover (*Charadrius marginatus*) and Kittlitz's plover (*Charadrius pecuarius*) in the area, following the methodology of the Kentish plover guide (Szekely et al., 2008). Finally, a bird survey of the Andavadoaka region and the island of Nosy Hao was conducted in different habitats (spiny forest, euphorbia scrubs, salt-marshes, and coasts).





**Figure 1.** Map of the Andavadoaka salt marshes used by more than 50 individuals of Kittlitz's plovers as breeding site (highlighted with blue).

## Results

### *Experimental Kittlitz's plovers*

In total 36 Kittlitz's plovers were captured, measured and ringed for the proposed experiment (18 males and 18 females) in three different salt-marshes (Fig. 1). Blood samples and ornamentation pictures of each individual were taken for sexing them (Fig. 2). The biometric measures of adult plovers are given in table 1.

### *Nests and eggs of the experimental Kittlitz's plovers*

One pair was included in the experiment when their nest was destroyed by a cow and the mate divorced finding new mates. 16 nests were found with a clutch size of two eggs, and one nest was found with one egg in advanced state of incubation (10 day approximately). A total of 33 eggs were relocated in new nests with almost the same state of incubation. Five Madagascar plover's nests and 28 Kittlitz plover's nests were used as new nests for the eggs of the experimental plovers.

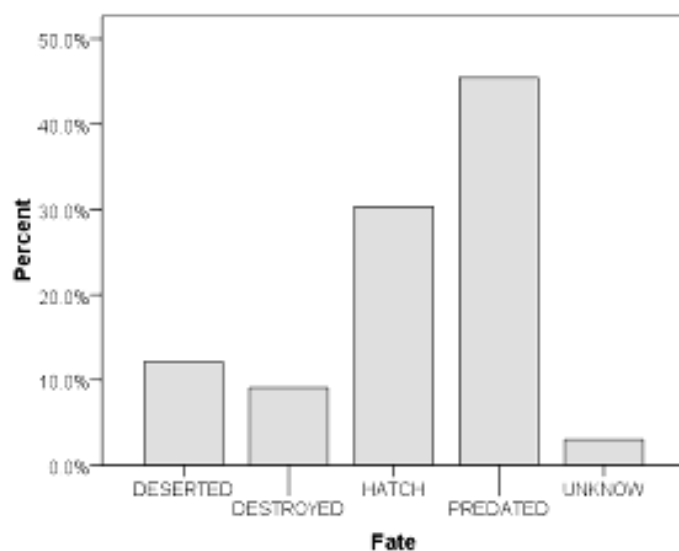
The experimental plover's eggs fate in their new nests is showed in the figure 3 and the biometric measures of the eggs are given in the table 2.



**Figure 2.** Blood sampling of adult Kittlitz's plover (left). Adult Kittlitz's plover photographed to measure ornamentation (right).

**Table 1.** Biometric descriptive statistics of 36 experimental Kittlitz's plovers.

Variable	Mean	Std. Deviation
Weight (g)	35.12	±2.67
Wing (mm)	105.31	±4.06
Tarsus (mm)	31.41	±1.12
Bill (mm)	17.96	±1.19



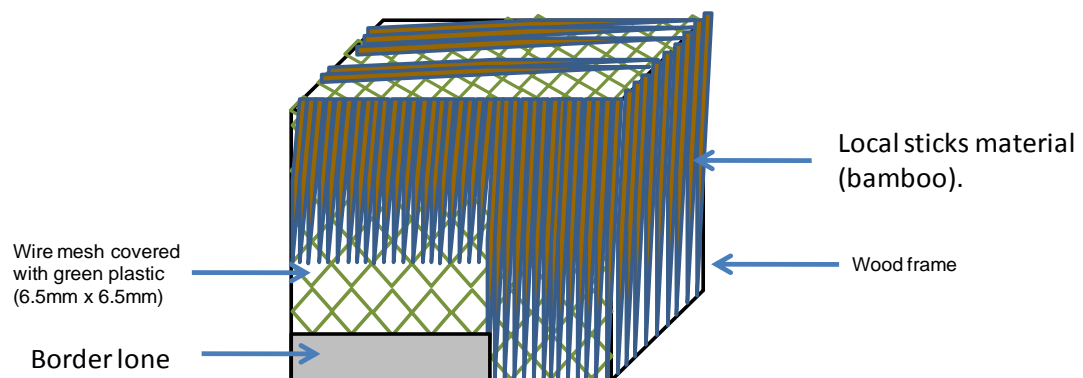
**Figure 3.** Fate of 33 eggs relocated in new nests found between February and April 2010 in Andavadoaka salt-marshes.

**Table 2.** Kittlitz's plover's eggs biometric descriptive statistics  $n = 18$ .

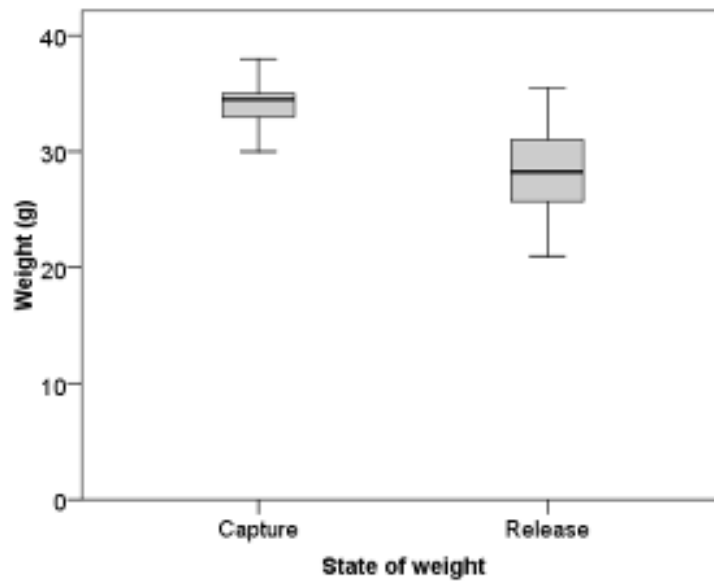
Variable	Mean $\pm$ SD
Length of egg (mm)	31.23 $\pm$ .996
Breath of egg (mm)	22.06 $\pm$ .570

*Captive Kittlitz's plover*

An aviary of 4 cages (1m x 1.5m each) was built with standard and local materials (Fig 4). Each cage had two washing dishes with clean water and two feeding dishes, which were cleaned every day. 17 individuals of Kittlitz's plover were maintained in captivity. 1 individual died in captivity. The mean days of captivity was 6.81 ( $n=16$ ,  $sd \pm 3.82$ ). There were a significantly difference in weight of the plovers at the moment of capture which was higher than the weight at the moment of released from captivity (Fig. 5, Paired-samples t-test,  $t = 7.778, n = 17, p = .0001$ ).



**Figure 4.** Materials and size of each cage built to maintain an individual of Kittlitz's plover. Pictures of one cage and the aviary.

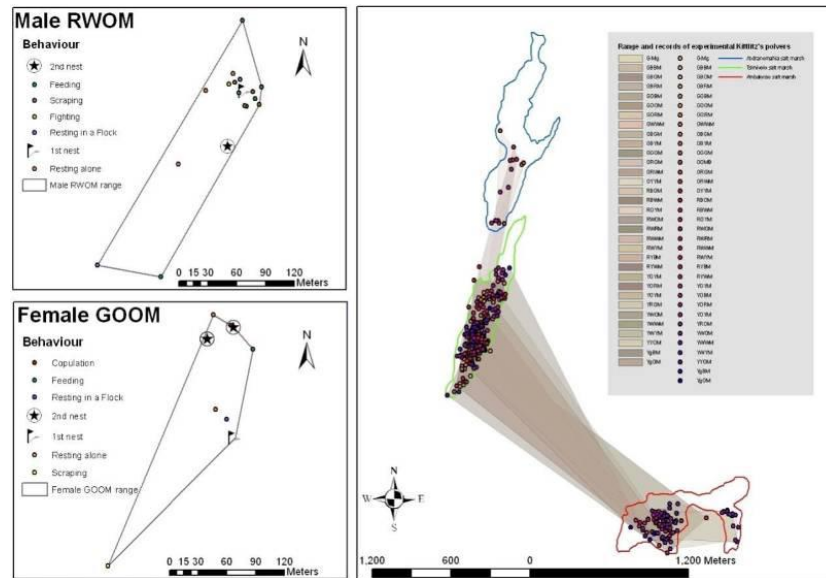


**Figure 5.** Weight of 17 experimental Kittlitz's plovers at the moment of capture and release from captivity.

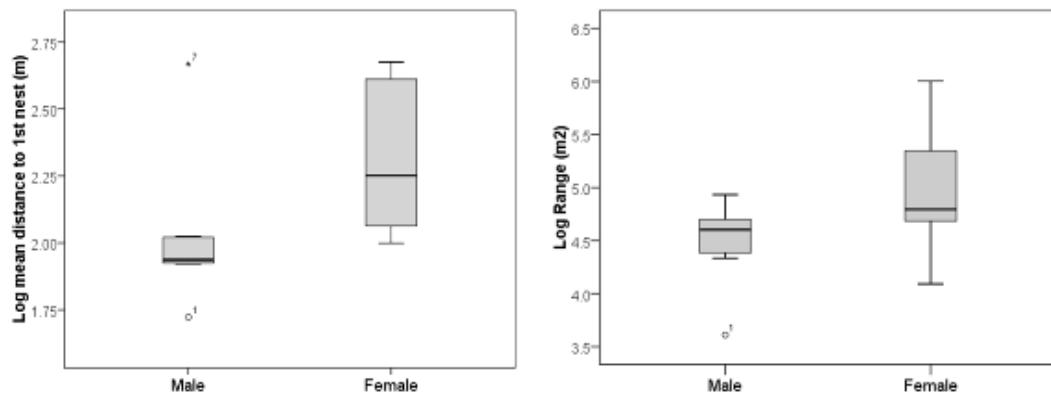
*Movements and mating behaviour of Kittlitz's plover*

In total, 531 records of behaviours and locations were taken of the experimental plovers after released at manipulation (18 individuals) and from captivity (16 individuals). It was calculated the area of action (range) of 33 individuals on the salt marshes during the breeding season. The movements and range of the individuals are shown in the figure 6.

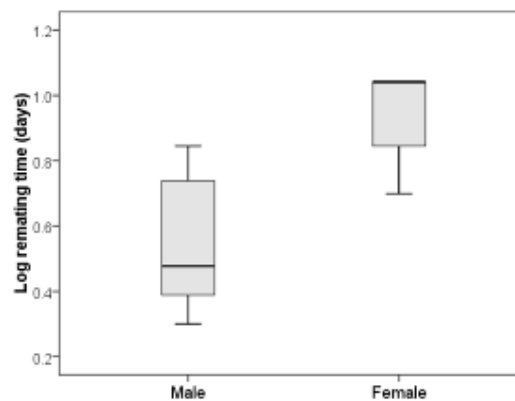
The sex of 6 males and 7 females was established when they copulated with this new mate. It was used these data to know trends in the mating behaviour and movements of the sexes. Males stayed nearer to their previous territory and used smaller areas of action than females (Fig. 7, Mann-Whitney  $U$  test Mean distance previous territory,  $Z = -1.981$ ,  $p = .048$ ; Mann-Whitney  $U$  test range,  $Z = -1.597$ ,  $p = .110$ ). In contrast to the expected mating times, males remated more quickly (median = 3.5, range: 2-6,  $n = 6$ ) than females (Fig. 8, median = 9, range: 5-11,  $n = 5$ , Fig. 8, Mann-Whitney  $U$  test,  $Z = -2.388$ ,  $p = .017$ ).



**Figure 6.** Map shows movements and ranges of 33 individuals of Kittlitz's plover in Andavadoaka salt marshes (right). Two example of the range and behaviours of one male and one female of experimentally deserted Kittlitz's plovers (left).



**Figure 7.** The mean distance from previous territory and area of action –range- of males and females of experimental deserted Kittlitz's plovers.



**Figure 8.** Remating time of male and female Kittlitz's plover.

### Breeding ecology of Malagasy plovers

#### *Captures*

In total 138 plovers of three species were captured and sampled: 89 adults and 9 juveniles of Kittlitz's plover (*Charadrius pecuarius*); 22 adults and 8 juveniles of Madagascar plover (*Charadrius thoracicus*), and 10 adults of White-fronted plover (*Charadrius marginatus*). The biometric measures of adult and juvenile plovers are given in table 3.

**Table 3.** Biometrics of captured adults and juveniles of plovers in Andavadoaka

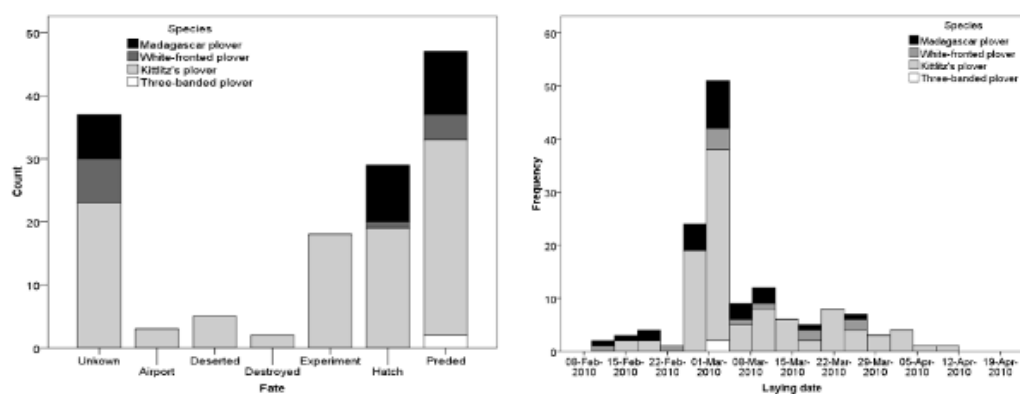
Plover species	Age	Mean $\pm$ SD Tarsus (mm)	Mean $\pm$ SD Wing (mm)	Mean $\pm$ SD Weight (g)	n
Kittlitz	Adult	31.48 $\pm$ 1.15	105.76 $\pm$ 3.40	34.93 $\pm$ 2.29	89
	Juvenile	20.73 $\pm$ 1.58		6.72 $\pm$ 1.02	9
Madagascar	Adult	32.67 $\pm$ 1.06	108.27 $\pm$ 3.95	36.90 $\pm$ 2.76	22
	Juvenile	22.60 $\pm$ 5.64	105.00	9.85 $\pm$ 6.55	8
White-fronted	Adult	26.22 $\pm$ 1.53	104.80 $\pm$ 3.82	37.10 $\pm$ 2.32	10

#### *Nests*

The number of nest found and their fate are shown in the table 4 and the figure 9 respectively. The laying date had a peak at the beginning of March and started to decrease through April (Figure 9). Kittlitz's plover was the specie with major number of nests followed by Madagascar plover, White-fronted plover and 2 nests of Three-banded plover. The laying date per species is given in the figure 9 and pictures of chicks in the figure 10.

**Table 4.** Number of nests per species found in the salt marshes around Andavadoaka. KTP: *Charadrius pecuarius*, MP: *Charadrius thoracicus*, TCP *Charadrius tricollaris* and WFP *Charadrius marginatus*.

Site	KTP	MP	TCP	WFP	Total
Andranomahia	14	6		2	22
Andranomasaka	21	3	2	1	27
Anjinalambezo		4			4
Antsirabe east	1	1			2
Antsirabe North	10	3		1	14
Antsirabe southeast	1	2			3
Antsirabe west	2	1		3	6
Beloba	22	2		2	26
Coco beach				1	1
Manga Beach				1	1
Tsimivolo	22	5			27
<b>Total</b>	<b>99</b>	<b>27</b>	<b>2</b>	<b>12</b>	<b>140</b>



**Figure 9.** Count of fate per species in the salt marshes of Andavadoaka (left). Estimated laying dates of 140 nests of 4 species plovers found between February 21st and April 14th at Andavadoaka (right).





**Figure 10.** Chicks and eggs of Madagascar plover *Charadrius thoracicus*.

#### *Bird survey in Andavadoaka Area*

The region of Andavadoaka is of special biological interest due to the occurrences of many resident and migratory water and terrestrial birds (Table 5). Four vulnerable species and one near threatened were recorded: Long-tailed Ground-roller *Uratelornis chimaera*, Subdesert Mesite *Monias benschi*, Humblot's heron *Ardea humbloti*, Madagascar plover *Charadrius thoracicus*, and Reunion harrier *Circus maillardi*. The area also provides refuge to 17 recorded migratory bird species most of them waterbirds. Figure 11 and 12 show some pictures of the birds of Andavadoaka.

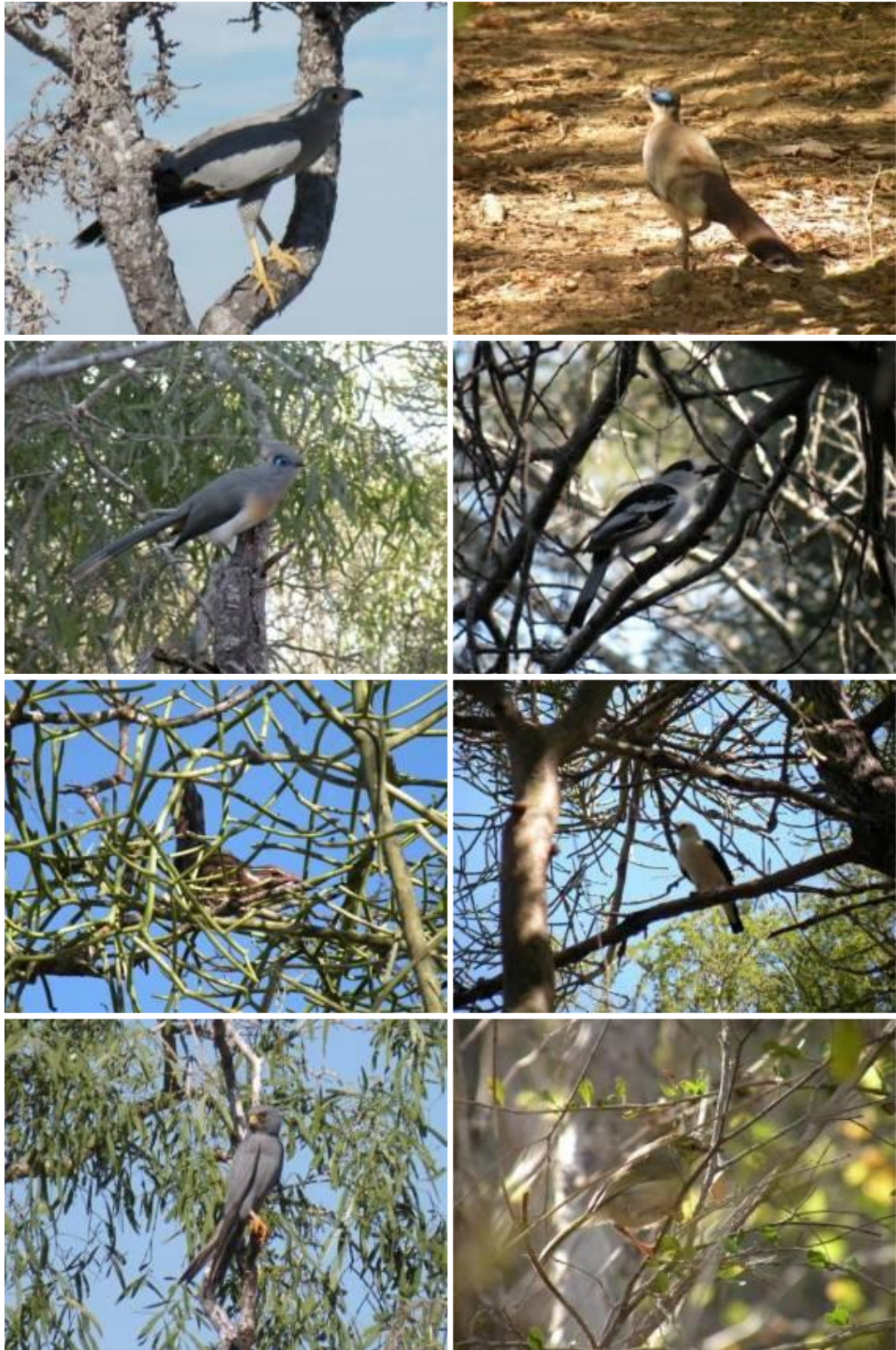


**Table 5.** Bird list of the species recorded in Andavadoaka region between February to May 2010.

N	Species name	English name	Conservation status	Habitat
1	<i>Nycticorax nycticorax</i>	Black-crowned night heron	Least concern	Wetlands
2	<i>Bubulcus ibis</i>	Cattle egret	Least concern	Grasslands / wetlands
3	<i>Butorides striatus</i>	Green-backed heron	Least concern	Wetlands
4	<i>Egretta ardesiaca</i>	Black egret	Least concern	Wetlands
5	<i>Egretta dimorpha</i>	Dimorphic egret	Least concern	Wetlands
6	<i>Egretta alba</i>	Great egret	Least concern	Wetlands
7	<i>Ardea cinerea</i>	Grey heron	Least concern	Wetlands
8	<i>Ardea humbloti</i>	Humblot's heron	<b>Vulnerable</b>	Wetlands
9	<i>Phoenicopterus ruber</i>	Greater flamingo	Least concern	Wetlands
10	<i>Anas erythrorhyncha</i>	Red-billed teal	Least concern	Wetlands
11	<i>Pandion haliaetus</i>	Osprey	Least concern	Wetlands / coasts
12	<i>Polyboroides radiates</i>	Madagascar harrier-hawk	Least concern	Forest
13	<i>Circus maillardi</i>	Reunion harrier	<b>Near threatened</b>	Marshy edges
14	<i>Accipiter francesii</i>	Frances's sparrowhawk	Least concern	Forest
15	<i>Buteo brachypterus</i>	Madagascar buzzard	Least concern	Forest
16	<i>Falco newtoni</i>	Madagascar kestrel	Least concern	Grasslands / wetlands
17	<i>Falco zoniventris</i>	Banded kestrel	Least concern	Forest edges
18	<i>Falco eleonora</i>	Eleonora's falcon	Least concern	Marshy edges
19	<i>Falco concolor</i>	Sooty falcon	Least concern	Marshy edges
20	<i>Margaroperdix madagascarensis</i>	Madagascar partridge	Least concern	Bushy areas and grasslands
21	<i>Numida meleagris</i>	Helmeted guinea fowl	Least concern	Forest edges / grasslands
22	<i>Mesiternis benschi</i>	Subdesert mesite	<b>Vulnerable</b>	Dry, spiny forest
23	<i>Turnix nigricollis</i>	Madagascar buttonquail	Least concern	Grasslands / dry forest
24	<i>Fulica cristata</i>	Red-nobbed coot	Least concern	Wetlands
25	<i>Dromas ardeola</i>	Crab plover	Least concern	Coasts
26	<i>Himantopus himantopus</i>	Black-winged stilt	Least concern	Coasts
27	<i>Charadrius hiaticula</i>	Ringed plover	Least concern	Wetlands
28	<i>Charadrius pecuarius</i>	Kittlitz's plover	Least concern	Wetlands
29	<i>Charadrius thoracicus</i>	Madagascar plover	<b>Vulnerable</b>	Wetlands
30	<i>Charadrius tricollaris</i>	Three-banded plover	Least concern	Wetlands
31	<i>Charadrius marginatus</i>	White-fronted plover	Least concern	Wetlands
32	<i>Charadrius leschenaultia</i>	Greater sand plover	Least concern	Wetlands
33	<i>Limosa lapponica</i>	Bar-tailed godwit	Least concern	Wetlands
34	<i>Numenius phaeopus</i>	Whimbrel	Least concern	Wetlands
35	<i>Numenius arquata</i>	Eurasian curlew	Least concern	Wetlands
36	<i>Tringa stagnatilis</i>	Marsh sandpiper	Least concern	Wetlands
37	<i>Tringa nebularia</i>	Common greenshank	Least concern	Wetlands
38	<i>Tringa glareola</i>	Wood sandpiper	Least concern	Wetlands
39	<i>Xenus cinereus</i>	Terek sandpiper	Least concern	Wetlands
40	<i>Actitis hypoleucos</i>	Common sandpiper	Least concern	Wetlands
41	<i>Arenaria interpres</i>	Ruddy turnstone	Least concern	Wetlands
42	<i>Calidris alba</i>	Sanderling	Least concern	Wetlands
43	<i>Calidris ferruginea</i>	Curlew sandpiper	Least concern	Wetlands
44	<i>Sterna bengalensis</i>	Lesser crested tern	Least concern	Coasts
45	<i>Pterocles personatus</i>	Madagascar sandgrouse	Least concern	Grasslands / wetlands
46	<i>Streptopelia picturata</i>	Madagascar turtle dove	Least concern	Forest
47	<i>Oena capensis</i>	Namaqua dove	Least concern	Grasslands / wetlands
48	<i>Treron australis</i>	Madagascar green pigeon	Least concern	Forest
49	<i>Coracopsis vasa</i>	Greater vasa parrot	Least concern	Forest
50	<i>Agapornis canus</i>	Grey-headed Lovebird	Least concern	Grasslands / wetlands
51	<i>Cuculus rochii</i>	Madagascar lesser cuckoo	Least concern	Forest
52	<i>Coua ruficeps olivaceiceps</i>	Red-capped coua	Least concern	Dry, spiny forest
53	<i>Coua cristata</i>	Crested coua	Least concern	Dry, spiny forest

# APPENDIX ONE

N	Species name	English name	Conservation status	Habitat
54	<i>Centropus toulou</i>	Madagascar coucal	Least concern	Dry, spiny forest
55	<i>Caprimulgus madagascarensis</i>	Madagascar nightjar	Least concern	Grasslands
56	<i>Cypsiurus parvus</i>	African palm swift	Least concern	Coasts / wetlands
57	<i>Merops superciliosus</i>	Madagascar bee-eater	Least concern	Grasslands / wetlands / open habitats
58	<i>Uratelornis chimaera</i>	Long-tailed ground-roller	<b>Vulnerable</b>	Dry, spiny forest
59	<i>Leptosomus discolor</i>	Cuckoo-roller	Least concern	Dry, spiny forest
60	<i>Upupa marginata</i>	Madagascar hoopoe	Least concern	Dry, spiny forest edges
61	<i>Mirafra hova</i>	Madagascar Bush lark	Least concern	Grasslands / wetlands / open habitats
62	<i>Hirundo rustica</i>	Barn swallow	Least concern	Wetlands
63	<i>Hypsipetes madagascariensis</i>	Madagascar bulbul	Least concern	Forest
64	<i>Calicalicus rufocarpalis</i>	Red-tailed vanga	Least concern	Forest
65	<i>Vanga curvirostris</i>	Hook-billed vanga	Least concern	Forest
66	<i>Xenopirostris xenopirostris</i>	Lafresnaye's vanga	Least concern	Spiny forest and coastal euphorbia scrub
67	<i>Xenopirostris damii</i>	Van Dam's vanga	<b>Vulnerable</b>	Forest
68	<i>Falco pinnatus</i>	Sickle-billed vanga	Least concern	Spiny forest and coastal euphorbia scrub
69	<i>Leptopterus chabert</i>	Chabert's vanga	Least concern	Spiny forest and coastal euphorbia scrub
70	<i>Copsychus albospectus</i>	Madagascar Magpie-robin	Least concern	Forest
71	<i>Nesillas lantzii</i>	Subdesert brush warbler	Least concern	Euphorbia forest
72	<i>Thamnornis chloropetoides</i>	Thamnornis warbler	Least concern	Spiny forest and coastal euphorbia scrub
73	<i>Newtonia brunneicauda</i>	Common newtonia	Least concern	Spiny forest and coastal euphorbia scrub
74	<i>Cisticola cherina</i>	Madagascar cisticola	Least concern	Grasslands / wetlands / open habitats
75	<i>Neomixis tenella</i>	Common jery	Least concern	Spiny forest and coastal euphorbia scrub
76	<i>Neomixis striatigula</i>	Stripe-throated jery	Least concern	Spiny forest and coastal euphorbia scrub
77	<i>Nectarinia souimanga</i>	Souimanga sunbird	Least concern	Forest
78	<i>Lonchura nana</i>	Madagascar mannikin	Least concern	Grasslands / wetlands / open habitats
79	<i>Ploceus sakalava</i>	Sakalava weaver	Least concern	Grasslands / wetlands / open habitats
80	<i>Foudia madagascariensis</i>	Madagascar red fody	Least concern	Grasslands / wetlands / open habitats
81	<i>Acridotheres tristis</i>	Common myna	Least concern	Open habitats
82	<i>Dicrurus forficatus</i>	Crested drongo	Least concern	Grasslands / wetlands edges
83	<i>Corvus albus</i>	Pied crow	Least concern	Open habitats



**Figure 11.** From the top (left to right): Madagascar harrier-hawk (*Polyboroides radiates*), Red-capped coua (*Coua ruficeps*), Crested coua (*Coua cristata*), Hook-billed vanga (*Vanga curvirostris*), Subdesert mesite (*Mesiternis benschi*), Sickie-billed vanga (*Falculea palliate*), Sooty falcon (*Falco concolor*), Common jery (*Neomixis tenella*).





**Figure 12.** From the top (left to right): Ringed plover (*Charadrius hiaticula*), Sanderling (*Calidris alba*), Madagascar and White-fronted plovers (*Charadrius thoracicus* and *Charadrius marginatus*), Kittlitz's plover (*Charadrius pecuarius*), Madagascar plover with chick (*Charadrius thoracicus*), Madagascar hoopoe (*Upupa marginata*), Madagascar bee-eater (*Merops superciliosus*), Common jery (*Neomixis tenella*).

## Discussion

The fieldwork was very successful to test proposed prediction about mating opportunities of the population of Kittlitz's plover in Andavadoaka. Blood samples, ornamentation, morphology, movements, behaviours, and re-mating times were taken to tests hypothesis of the evolution of the breeding systems. Some preliminary trends emerge from the individuals of Kittlitz's plover already sexed in field. Males are more territorial than females as expected in plovers populations (Szekely et al., 1999). Males after stimulation returned to their small territories where they had their nests, while females moved far away from the same point.

Furthermore, the same sexed individuals in field showed a trend in the mating opportunities measured by the re-mating time. Females spend more time finding new mates than males, which is completely different in other species of plover where is reversed (Szekely et al., 1999). However, it is necessary finishing the molecular analysis, for sexing the entire individuals, to know if the trends remain similar.

The area of Andavadoaka has also a great population of Madagascar plover and White-fronted plover to study the breeding ecology of these species. The peak of the breeding season for all the community of plovers was at the end of February and at the beginning of March. It coincided with the end of the rain season and the start of the dry season (personal observations for 2010). Most of the nests were predated by natural predators and domestic animals (dogs and cats) which are a great pressure on the populations of plovers and a consequence for the conservation of the vulnerable Madagascar plover in the area. The area has special characteristics such as big populations of plovers; preserve natural habitats, facilities for field working to continue a monitoring programme of plovers to study their evolution, ecology, behaviour and conservation.

Finally, the area is of special biological interest due to the occurrences of many resident and migratory water and terrestrial birds. The salt marshes in Andavadoaka are important habitats of plover populations and specifically for the endemic and vulnerable Madagascar plover *Charadrius thoracicus*. However, one of the best areas used for nesting was completely destroyed by the construction of an airport. This area, known as Tsimivolo salt marsh, was home of almost 40 individual of the Madagascar plover.

In the region, three other vulnerable species have been recorded: the Long-tailed Ground-roller *Uratelornis chimaera*, the Subdesert Mesite *Monias benschi*, and Humblot's heron *Ardea humbloti*, and one near threatened species the Reunion harrier *Circus maillardi*, which are endangered because of the destruction of their habitat causing significant population declines. The area also provides refuge to 17 recorded migratory bird species most of them waterbirds. The Andavadoaka region meet several RAMSAR criteria (The Convention on Wetlands of International Importance), which must be studied in depth to inform conservation and wise use of this habitat.

The conservation of the salt-marshes are important for the dynamics of the ecological process in surrounding habitats such as mangroves, sea grasses, coral reefs, Euphorbia forests, spiny forests and most importantly, for the ecological services offered to the local community.

### **Acknowledgement**

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## APPENDIX TWO

### **Sex roles, breeding systems and mate availability in Malagasy plovers**

Fieldwork Report

1 April – 23 June 2011

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## Summary

Sex role behaviour might be influenced by variation in adult sex ratios, thus uniparental care should emerge when the adult sex ratio is biased and one sex has higher mating opportunities. Between 1<sup>st</sup> April and 23<sup>rd</sup> June of 2011 was carried out a mate removal experiment to test this proposition with 14 pairs of White-fronted plover in the South-west coast of Madagascar. Plovers found a new mate quickly and it seems that the mating time is similar for males and females. However, molecular sexing is needed to confirm the pattern. Additional data were taken to describe patterns in brood attendance, chick crypsis and demography in white-fronted, Kittlitz's and Madagascar plovers. Male and female White-fronted plovers were observed attending the brood in all cases at different chick age states. For the Kittlitz's plover only one parent was observed attending the brood in all cases at different chick age states. The sex of the attending parent in Kittlitz's plovers will be identified by molecular sexing techniques. Fieldwork data will be analysed for testing hypotheses related with breeding systems evolution and sex ratios.

## Résumé

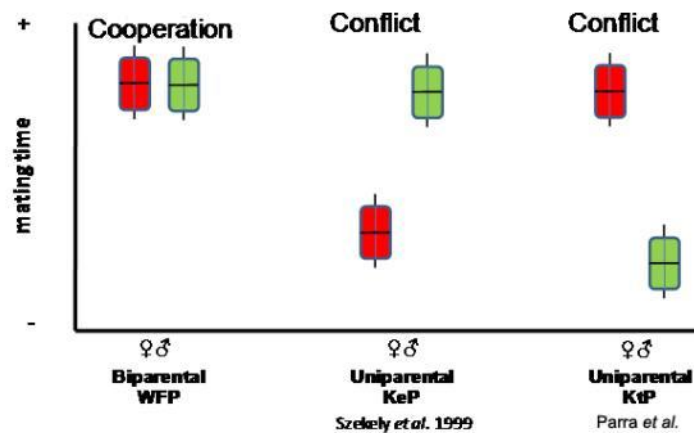
Le rôle du comportement sexuel pourrait être influencé par des variations entre le rapport entre le nombre des adultes mâles et femelles (sexe ratio), ainsi le soin des petits doit être pris uniquement par l'un des parents lorsqu'il y a un déséquilibre entre ce rapport et que l'un des parents a plus d'opportunité de s'accoupler avec des autres partenaires. Entre le 1er avril et le 23 Juin 2011, une expérience sur la séparation des couples pour tester cette hypothèse avec 14 couples de Pluvier pâtre a été réalisée dans la côte sud-ouest de Madagascar. Chaque individu du couple a trouvé rapidement un nouveau compagnon après la séparation et il semble que le temps de trouver le nouveau partenaire semble identique pour les mâles et les femelles. Toutefois, la détermination du sexe de chaque partenaire par des analyses moléculaires est nécessaire pour confirmer cette tendance. Des données supplémentaires ont été prises pour décrire les tendances sur le type des soins des petits, la croissance des petits et la démographie des Pluviers pâtres, Pluviers de Kittlitz et le Gravelot de Madagascar. Les deux parents (mâle et femelle) ont été vus ensemble à attendre leurs petits pendant les différents stades de développement des petits. Pour le Pluvier de Kittlitz, seulement un parent a été vu à attendre les petits pendant son développement. Le sexe du parent de Pluvier de Kittlitz attendant les petits sera à déterminer ultérieurement à l'aide des analyses moléculaires. Les données seront à analyser pour tester les hypothèses relatives à l'évolution des systèmes de reproduction et de sexes ratios.

## Introduction

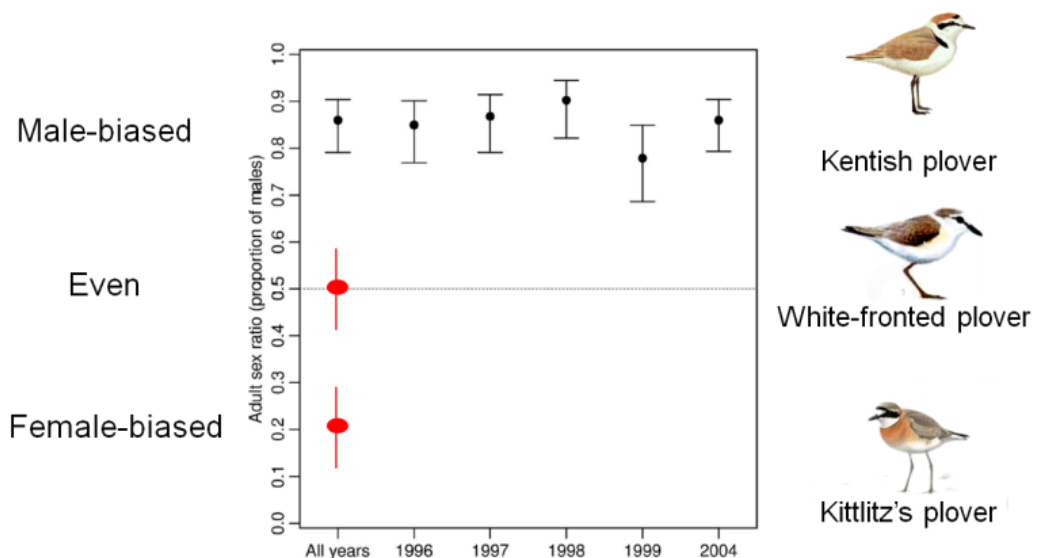
There is a complex relationship among mating systems, parental care and adult sex ratios, and theoretical and experimental studies are beginning to tease apart their interactions (Queller 1997, Kokko and Jennions 2003, Kokko, and Jennions 2008). For example, Kentish plovers *Charadrius alexandrinus* have male biased parental care, and theory predicts this should emerge when the adult sex ratio is male-biased, thus females have higher mating opportunities (Barta et al. 2002, McNamara et al. 2000, Jennions and Kokko 2010). An experimental test of the latter proposition found that male plovers spent more time finding a new mate than the female plovers (Székely et al. 1999). In 2010, I found that female Kittlitz's plovers *Charadrius pecuarius*, remated substantially more slowly than males. This result is striking because it is the opposite to what was found in the Kentish plover (Székely et al. 1999). These results suggest that the ASRs are different between these two species: female-biased ASR in the Kittlitz's plover whereas male-biased ASR in the Kentish plover. This extreme difference in ASR between closely related species is striking, and it suggests that sex roles may evolve in response to ASR as predicted by theoretical models.

Here, I repeated the experiment in a common monogamous species, the White-fronted plover *Charadrius marginatus*, to assess the mating time spends in finding a new mate. We predict that male and female White-fronted plover will spend more time finding a new mate after divorce than polygamous species (Fig. 1). Experimental deserting male and female of plovers were used to estimate the mating time and to measure defence of territorial resources and site fidelity. Additional fieldwork data were took to describe patterns in brood attendance and chick crypsis in relation with their environment in Kittlitz's and white-fronted plovers, to understand how mating opportunities and ASR have been originated in these two plover species. Finally, individuals of Kittlitz's, white-fronted and Madagascar plovers were resighted and colour ringed for future demographic study of the expected ASR of these species (Fig. 2).

## Predictions



**Figure 1.** Expected mating times in three species of plovers with different brood care behaviour: WFP, white-fronted plover, KeP, Kentish plover and KtP Kittlitz's plover.

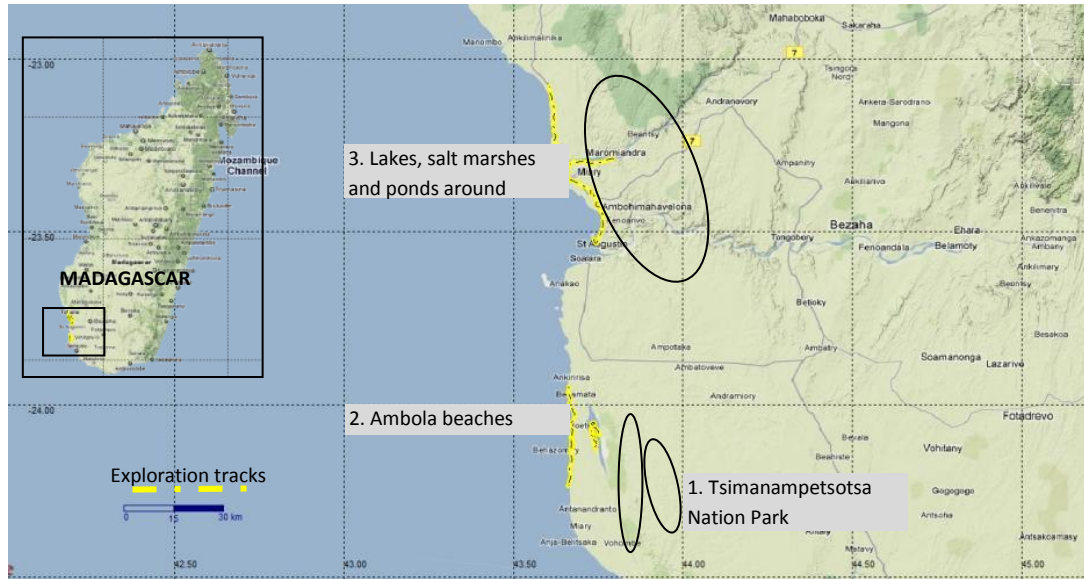


**Figure 2.** Expected adult sex ratios in two species of plover from Madagascar (red points) in contrast to Kentish plover (Kosztolányi et al. 2011).

## Methods

Study sites: Fieldwork was carried out at three sites in the South-west Madagascar where population of White-fronted, Kittlitz's, Madagascar and Three-banded plovers were found (Fig. 3): 1) Tsimanampetsotsa National Park (Lake Tsi, 24° 3'51.70"S, 43°44'21.96"E) is a salt lake for about 15 km long by 0.5 km width surrounded by

spiny forest, grassland habitats and sandy shores (Fig. 4); 2) Ambola beaches are approximately 30 km of sandy sea shore to north and south forward of Ambola village in the south coasts of Madagascar ( $24^{\circ} 3'2.28''\text{S}$ ,  $43^{\circ}40'30.17''\text{E}$ ). Euphorbia coastal scrubs, Australian pines on the beaches and white sandy beaches are the main habitats of this site (Fig. 5); and 3) Lakes, salt marshes and ponds around the city of Toliara ( $23^{\circ}24'22.24''\text{S}$ ,  $43^{\circ}43'25.96''\text{E}$ ). These areas are surrounded by mud, grass, palms and spiny forest habitats (Fig. 6).



**Figure 3.** Map of study sites in the South-west Madagascar.



**Figure 4.** Lake Tsimanampetsotsa National Park



**Figure 5.** Ambola beaches



**Figure 6.** Andasibe – Lake Asingo

White-fronted plover mating opportunities: Field methods followed the Kentish plover guide (Székely et al. 2007). Two round traps were used to catch the pair of 14 nests of White-fronted plovers randomly chosen on the shore of Lake Tsi. Morphological traits (body mass, tarsus length and wing length) were measured with a sliding calliper, wing ruler, and spring balance. Blood samples and ornamentation pictures were also taken from each individual following the practical guide to photograph ornaments of plovers (Argüelles-Ticó 2008). Additionally, each individual were ringed with an individual combination of colour rings and SAFRING metal ring (Fig. 7).

The experiment was similar to Székely et al. (1999) with the Kentish plover in Turkey. One parent (male or female) was released randomly at manipulation before its flanks were dyed with green marker. The other parent was taken into an individual aviary built next to the fieldwork camp (7 cages of 1m height x 1.5 length x 1.5 width, Fig. 8), and only released after their mate had found a new mate or after 2 weeks of captivity. White-fronted plover is sexually monomorphic then at the time of the manipulation we did not know the sex of the released plovers. The clutch of eggs was distributed into other plover nests. They were checked every 4 days in their new nests.

Captive plovers were fed with appropriate food and drinks twice per day every day to maintain their good health using a high protein dry insects, worms, shrimps, fish and supplement vitamins in the water. They were released after their former mate found a new partner or after 12 days of captivity. Before release, each plover was





**Figure 8.** Aviary built next to the camp site. Left cages without cover, right aviary covered with piñon plants and surrounded with spine shrubs.

measured and marked by green marker. The released individuals were searched every day following the same methodology as above.

Kittlitz's and white-fronted plover parental care and chick camouflage patterns:  
Colour ringed families were visited every two days and recorded the number and colour rings of attending parents and the number of chicks. Chicks were photographed after their hid in their environment as follow: after spot a chick, from a car with spotting scope or binoculars, one observer ran to capture the chick, as an event of predation, and other observe followed the chick to its hiding site. Marked and no-marked broods were colour and metal ringed, tarsus and weight measured, blood sampled and photographed in their environment with a Kodak gray card, 3 colour rings and a rule (Fig. 9 and 10).



**Figure 9.** White-fronted plover chick crypsis study. Left picture: chick hides on sandy and litter habitat (background). Right picture: chick on gray card board.





**Figure 10.** Kittlitz's plover chick crypsis study. Left picture: chick hides on muddy and grassland habitat (background). Right picture: chick on gray card board.

Malagasy plover demographic data: Nests of white-fronted, Kittlitz's, Madagascar and Three-banded plovers were searched in the three study sites by looking the behaviour of the parents or parent. Eggs on nests were measuring as above. Round and funnel traps were used to catch the pair of plovers on the nests or broods found at the three sites. Morphological traits (body mass, tarsus length and wing length) were measured to adult plovers with a sliding calliper, wing ruler, and spring balance. Blood samples and ornamentation pictures were also taken from each individual following the practical guide to photograph ornaments of plovers (Argüelles-Ticó 2008). Additionally, each individual were ringed with an individual combination of colour rings and SAFRING metal ring. Finally, colour ringed individuals spotted throughout the field study sites were geo-positioned with a GPS device and recorded its behaviour, pair-bond, date, time and habitat.

## Results

White-fronted plover mating opportunities: 14 nests of white-fronted plover were used in the mating time experiment. In total 14 pairs (28 adults plovers) were manipulated. 14 colour ringed parents (male or female) were released after measured them (at manipulation) and their 14 formal mates were taken into captivity (after manipulation). From 14 plovers released at manipulation: 12 found a new mate (mean mating time 3.24 days, std. deviation +/- 1.35 days, minimum 1 day– maximum 5 days); and two did not find a new mate after 14 days. From 14 plovers kept in captivity: 12 recovered their formal mates and territories after fight against rivals (the same released day), 1 found a new mate and 1 died in captivity.

## APPENDIX TWO

Behavioural samples were taken to 25 manipulated plover. 52 hours of behavioural samples were taken to 13 plovers released at field and 17.6 hours of behavioural samples were taken to 12 plovers released from captivity (Table 1).

**Table 1.** Behavioural sample took to experimental adults white-fronted plovers: 1 mated with a new plover, 2 remated with its formal mate, and 3 unmated.

Plover	ID	Mated	Remated	Unmated	Total
At manipulation	MB/YY	61		61	122
	MG/BG	142	76	1	219
	MG/BY	498	153	121	772
	MG/GG	357	227	186	770
	MG/WG	304	92	38	434
	MG/WW	71	126	240	437
	MG/WY		64	345	409
	MG/YG	162	140	253	555
	MG/YY		122		122
	MY/BY	113	174		287
	MY/WW	272	215	185	672
	MY/YY	563	196	92	851
	RO/MY	304	242	35	581
	gg/MG		227		227
After manipulation	MG/BB		237		237
	MG/BW	61			61
	MG/GW		195	131	326
	MG/WB		126		126

Plover	ID	Mated	Remated	Unmated	Total
	MG/YW		100		100
	MY/BB		216		216
	MY/GB		122	49	171
	MY/WB		195	63	258
	OG/MY		63		63
	Rg/MW		142		142
	RR/MW		185		185
<b>Total</b>		<b>2908</b>	<b>3635</b>	<b>1800</b>	<b>8343</b>

Kittlitz's and white-fronted plover parental care and chick camouflage patterns: Four families of white-fronted plover and 14 families of Kittlitz's plovers were followed and recorded the number of attending parents and number of chicks. 9 chicks of white-fronted plover and 31 chicks of Kittlitz's plover were captured and photographed at the hiding places and on the gray card board.

Malagasy plover demographic data: 60 Malagasy plover nests and broods encounter are given by species in table 2. 92 Malagasy plover captures are given by species and age category in table 3. Finally, 27 colour ringed plovers from last year were resighted at Lake Tsi.

**Table 2.** Malagasy plover nests found in 4 sites of the South-west coast of Madagascar.

Species	Nest / Brood
Kittlitz's plover	30
Madagascar plover	1
Three-banded plover	1
White-fronted plover	28
<b>Total</b>	<b>60</b>

**Table 3.** Number of Malagasy plovers captured in 4 sites of the South-west coast of Madagascar.

Species	Adult	Chick	Juv	Total
Kittlitz's plover	18	24	7	49
Madagascar plover	1	2		3
Three-banded plover	1			1
White-fronted plover	30	9		39
<b>Total</b>	<b>50</b>	<b>35</b>	<b>7</b>	<b>92</b>

## Conclusions

### White-fronted plover mating opportunities

- 24 white-fronted plovers were used in the mating time experiment. Plover released at manipulation found a new mate very quickly and it seems the mating time is similar for males and females. However, molecular sexing is needed to confirm the pattern. Although, it was expected a longer mating time for male and female white-fronted plovers, the experiment might create breeding vacancies and mating opportunities for floating plover population. Thus, any mating opportunity and breeding territory vacancy should be taken for single plovers, as mates and territories might be a limited resource by high adult survival, mate fidelity and low dispersion (Lloyd 2008).
- Plover released from captivity recovered their formal mates and territories after fight against rivals in 12 cases. Mate fidelity could be explained as mates and territories might be the limited resource as well (Lloyd 2008).

### Kittlitz's and white-fronted plover parental care and chick camouflage patterns

- Male and female White-fronted plovers were observed attending the brood in all cases at different chick age states. This pattern is consistent with mate fidelity within and between years of the white-fronted plover and in highly monogamous species (Lloyd 2008).

- Only one parent of Kittlitz's plover was observed attending the brood in all cases at different chick age states. The sex of the parent will be identified by molecular sexing analyses.
- Patterns and colours of chicks camouflage in Kittlitz's and white-fronted plover will be described using photographic techniques as good numbers of pictures were taken during fieldwork. These patterns will be related with the environment, difference in chick mortality, sex ratios and breeding systems in plovers.

#### Malagasy plover demographic data

- There was no a peak in the breeding season for Malagasy plovers between April and June, as few number of nests and chick were found in this period. There were also no rains during this period and it could influence the reproductive behaviour of plovers. White-fronted plover was the species with more encounters of nests and broods in Lake Tsi. Early June some rains fell over the lakes and ponds around Tuleara city and broods and nests of Malagasy plovers were found.
- Demographic data of Malagasy plovers will be used to model chick and adult sex ratios to explain their breeding systems evolution.
- We found a new site for studying the breeding ecology of Malagasy plovers called Lake Asingo. The lake has a large number of aquatic species of birds including the four species of breeding plovers recorded in Madagascar. However, the lake is suffering pressure from pollution, hunting, over exploitation and drying. Critical conservation actions are needed to protect this sanctuary for aquatic wildlife.

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